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The Behavioural and Physical
Development of the
Athymic Nude Mouse

by

David Reginald Morgan

A Thesis
Submitted for the Degree of
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To My Family:

Anne, Lucy and Kate

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Abstract

A colony of outbred athymic nude mice was established using barrier maintenance systems. Heterozygous females were mated with homozygous nude males and the behaviour of the parents rearing mixed litters was examined using an observational time-sampling procedure. Longitudinal and cross-sectional behavioural and morphological studies on developing nude and hairy offspring were carried out from birth until weaning at day 28. Weaned mice who had not been subjected to any prior experimental situations were tested for exploratory behaviour in an open field.

Behavioural scan results indicate that heterozygous mothers display normal maternal behaviour and will build good nests in the proximity of the food/water containers. The male parent also demonstrated maternal behaviour. A high level of out-of-nest activity was found by the end of the second week post-partum, which correlated with cage activity and eating and drinking behaviour. Nude pups had difficulty in locating and attaching to the nipple and body weight curves confirmed that nude and hairy mice develop at different rates, even though nursed by the same dam. Litter manipulation experiments suggest that nudes benefit from the presence of hairy pups and that hairy pups could survive a 3 day period deprived of maternal care and milk and then successfully re-establish suckling when returned to the home nest. Ultrasonic calling experiments suggest that nude mice may produce more calls which are of longer duration than hairy mice when subjected to cold and isolation stress.

Nude mice appear retarded in their physical and behavioural development and do not explore an open field as actively as hairy pups at weaning. Retarded growth, a high incidence of neonatal mortality and alteration in behaviour may be related to early malnutrition and lack of later stimulation; the possible influences of athymia however, must also be considered.

CHAPTER 1

GENERAL INTRODUCTION

"The mouse mutant nude is likely to be extremely important in the immunology and in cancer research in the future. Already over 60 papers have been published describing the characteristics of the mutant, or making use of it as a tool in research. However, its widespread use has been partly reduced by its poor breeding performance and low viability under conventional conditions."

Dr. M.F.W Festing
MRC Laboratory Animals Centre
(1974)

This statement made by a leading authority on the husbandry techniques of nude mice (Festing & King, 1974) summarised the generally accepted problems of nude mice production in the early seventies.

By 1976 over 440 papers had been published, describing further, the characteristics and uses of this animal and in 1978 Fogh and Giovanella published "The Nude Mouse in Experimental and Clinical Research", which they considered to be the first major book on the subject.

Authors were always in general agreement that nude mice display a number of abnormal traits which affect body growth, weight gain, size, development, reproductive performance and longevity.

Nude Characteristics

Absence of hair

With the exception of sparse patches of hair that appear and disappear at sporadic intervals, homozygotes are devoid of hair.

Absence of Thymus

In homozygous fetuses, the thymus begins disgenesis at 14 days gestation. Neonatal mice display a 'thymus rudiment' only, and have few T-lymphocyte cells.

Susceptibility to disease and Longevity

Nude mice in conventional environments succumb easily to infection, display 'wasting disease' and begin to die between 3 and 4 weeks of age; such animals seldom live longer than 3 months.

Specific Pathogen Free (SPF) or Barrier maintained mice may reach 1 - 2 years of age.

Pre-weaning Mortality

Even under ideal conditions nude mice display a high incidence of pre-weaning mortality which may involve up to 50% of nudes born.

Poor growth and development

All nude mice, irrespective of genetic background, display a reduced body-growth rate. Homozygous neonates do not compete well with hairy sibs.

Poor reproductive performance

Some colonies exhibit poor reproduction, similar to inbred strains. Homozygous males and females may be sterile and females display poor maternal abilities and 'lactational failure' in many cases.

Nude mice from homozygous pairs seldom reach weaning age.

An outbred nude colony was established by the author in 1973 in conjunction with Ms Jill Oughton MRCVS at the Department of Veterinary Medicine at the University of Cambridge. By early 1975, the colony had greatly expanded was under the sole direction of the author and was becoming increasingly important as an experimental model in animal oncology studies.

The success of this colony is evidenced by a number of important publications which resulted during the ensuing five year period (see Appendix i) and by the fact that a number of laboratories, including the CRC Oncology Unit at Nottingham and the Houghton Poultry Research Station at St. Ives, Cambs, had initiated projects using these mice.

Although the colony continued to produce mice successfully, the unexplained phenomena of poor growth and development exhibited by nude offspring was always in evidence.

Previous work had failed to adequately study and explain the high incidence of perinatal mortality affecting nude pups; and of the factors affecting nutrition and growth curve of nudes compared to that of normal hairy sibs. No author had used behavioural tests to study the development of nudes and, probably because of the arduous and exacting maintenance programmes, little longitudinal growth data had been collected.

In 1977 therefore, I commenced a general behavioural and morphological study, using standard breeding animals. The objects of the work included:

1. To obtain cross-sectional and longitudinal data of body growth and weight gain in nude mice, when compared with normal hairy littermates.
2. To study some aspects of maternal behaviour by means of behaviour scan studies in natural and experimental nesting situations.
3. To carry out a number of litter manipulation experiments designed to investigate effects on nude mouse development, involving:
 - (i) Removal of some hairy pups from the litter at various ages post-partum.
 - (ii) Return of hairy pups to home cage after a 3 day separation from the litter and deprivation of maternal care.
4. To suggest when and why nude mice first display abnormal development.
5. To carry out a number of original behavioural experiments in order to characterise the behavioural development of nude mice, involving a study of:
 - 5.1 Ultrasonic calling.
 - 5.2 Development of Locomotion.
 - 5.3 Exploratory behaviour.

This study has attempted to review the relevant literature concerning behavioural and morphological development in the mouse and rat. As few references to such development in the nude mouse are available, the results of these experiments have been used to suggest some reasons for the abnormal growth and development cited, together with suggestions for further studies which, in the light of this work, could be usefully undertaken by other laboratories.

CHAPTER 2

(a) POSTNATAL BEHAVIOUR DEVELOPMENT

Introduction

Although development does appear to be more or less continuous, it does not proceed at a constant rate. This lack of constancy in the rates of developing systems gives rise to critical periods in development, based on the occurrence of major developmental processes.

The processes upon which the critical periods are dependent reflect the major forms of adaptation that occur at that time in development. Within a short time after birth the offspring's method of obtaining nutrition must change or must have changed. The change reflects a shift from the intrauterine to the extrauterine environment. Thus in non-precocial mammalian species there is an adaptation to sucking behaviour. This is only one of many forms of adaptation that exist during what has been called the neonatal period.

The critical periods of development in the mouse have been generally recognised and cited by Fox (1965): the neonatal period, the transition period, the pre-juvenile period, and the juvenile period, lasting from 26 days onward until sexual maturity. There follows an unlabelled period extending from the cessation of reproduction to the time of death.

Hinde (1966) in describing the influences which control the developing individual observed, "the determinants of the characteristics of the organism come from only two sources: the zygote and the

environment". Development then, depends upon the interaction between organism and environment. In the mouse for example, further modification in behavioural development may occur by the action of maternal care and nursing. Mice of the same genotype, kept within the same environment may show a difference in a particular trait, e.g. exploratory behaviour, which may be due to the amount of handling given by the parents to the young.

In rats and mice, age of pups, amount of externally induced stress and degree of stimulation from the young can affect the early mother-young interaction (Rosenblatt, 1975). Other factors affecting this relationship include, pup suckling ability (Findlay 1974; Teicher and Blass, 1976), size of the litter (Priestnall, 1972), milk ejection (Drewett and Trew, 1978), maternal nutrition (Smart and Silence, 1977) and olfactory and ultrasonic stimuli from the pups (Moore, 1981; Noirot, 1966).

Rodent studies have also shown some evidence for the existence of effective (Critical) periods for stimulation; for adult behaviour may be more influenced by a given type or degree of stimulation at one early stage of development than at another (Sluckin, 1970).

Studies on emotional reactivity (Denenberg, 1968; Archer, 1973), exposure learning (Hall, 1979) and exploratory behaviour (Thompson 1953 and 1956) have been reported in rodents and also, the influence of mutant genes (Denenberg et al., 1963; McClearn, 1964) and early nutrition, (MacDowell et al., 1930; Fox, 1965 and Eichenwald and Fry, 1969). It is clear that altered behaviour patterns in the adult, correlates with lack of stimulation and undernutrition in early life.

FOX: BEHAVIOURAL DEVELOPMENT OF THE MOUSE

Period	Duration	Main reflexological criteria
1. (Perinatal)	Birth - 3 days	Weak, rooting, righting, geotaxis and limb placing.
2. (Neonatal)	3 - 9	Strong and stereotyped reflexes and limb placing reactions, pivoting and circling locomotion.
3. (Postnatal) (Transition)	9 - 15	Primitive reflexes disappearing (e.g. rooting). Adult locomotor activities appear and organs of special sense are functional at the end of this period. Hyperkinesis disappear. Overgeneralized sensory responses seen.
4. Postnatal infantile (or Pre-juvenile)	15 - 26 days	Refinement of locomotor abilities and sensory capacities. Overgeneralized responses to sensory stimuli disappear.
5. Juvenile	26 days onward until sexual maturity	Further refinement of activities and sensory responses as the overgeneralized responsiveness is no longer seen and manipulative abilities are adult-like

Table 1 Critical periods in Mouse Development (from Fox, 1965).

In studying natural development or differences in behavioural development, a number of criteria have been used, depending on the animal, the area of development under study and the conditions prevailing. In the mouse for example, the maternal behaviour and mother-pup interactions, suckling/nursing behaviour, reflex development, emotional and exploratory behaviour are just a sample of the studies that have been undertaken.

Maternal Behaviour

Nest Building

The most common maternal responses of adult mice are nest building, nursing, retrieving and licking of the pups (Noirot, 1969_b). Maternal behaviour begins before the birth of the young under hormonal influence, as the pregnant female selects a suitable site at which to build a nest. The nest may have several functions but most importantly it offers thermal insulation to the poikilothermic newborn animals. The nest also offers concealment and acts as a barrier against straying when pup locomotory activity begins.

Any laboratory mouse or rat given strips of paper, wood shavings or cotton wool builds a small nest, which may be increased in size in response to low ambient temperature. Regardless of temperature, a female in late pregnancy builds a substantial "maternal" nest, which is maintained throughout the early postnatal period. However the young have an important influence on nest building and if a newborn litter of mice dies or is removed the mother reverts to building only a small nest (Richards, 1967).

The quality of nest produced by the mother may reflect the degree of maternal development, and can be assayed by descriptive means. Kaplan and Hyland (1972) rated a nest as 'good' if it had well-defined boundaries, was high, compact and made of well-shredded material. A nest rated 'adequate' still had definable but messy boundaries; it was flatter, more spread out and the material (paper) was not well-shredded. 'Poor' nests usually had no clearly definable boundaries, (often only a gradient of paper mixed with bedding) and the material was only coarsely shredded. Except for the period during pregnancy, the building of a maternal nest by a mouse depends on stimuli from the young. After about 14 days post-partum the nest-building activity declines as the pups develop locomotory powers and begin the transition from milk to solid food.

Nursing

Among all mammals the onset of maternal behaviour after parturition is rapid. This 'critical period' allows the mother and her newborn to form a behavioural bond that has important consequences for the young and without the formation of such a bond the young suffer in their development (Rosenblatt, 1975).

The onset of maternal behaviour is hormonally determined and it is most likely that the ovarian hormone oestradiol plays a major role under the specific conditions that exist pre-partum. After parturition however, maternal behaviour appears to be regulated chiefly by stimuli from the pups and hormones do not play a major role apart from their influence on lactation, (Rosenblatt, 1975). If a mother is separated from her litter during parturition after the young have been licked clean and afterbirths eaten, but before retrieving and nursing have taken place, she is unresponsive if the pups are returned on the fifth

day post-partum. Later on, a similar four day separation from the litter after the period of transition to dependence on pup stimulation has been completed, (i.e. after four days post-partum), may not produce this result.

In the absence of pup stimulation maternal responsiveness wanes rapidly but as little as 12 hours contact with pups post-partum maintains maternal responsiveness until the eighth day post-partum. Even 'naive' adult mice or rats will display maternal behaviour following a period of 'sensitisation'. In one study, of 115 naive mice (male and female) tested, nearly all showed some degree of maternal interest and 70% took up the lactation position when presented with young pups (Noirot, 1969b). Thus contact with pups exerts positive effects, since initially unresponsive animals after a sensitisation period, begin to retrieve and display other forms of maternal behaviour. The positive effects last for a few days.

Licking and Retrieving

From early life, small mammals of both sexes can be seen to comb, lick and scratch the body surface and during pregnancy, rats and mice increasingly lick the skin around the nipples and external genitalia. At parturition the licking is intensified and is accompanied by licking the young as they are born. Self-grooming in mice and rats is relatively stereotyped and self licking (face washing) may be observed from day 2 following birth (Bolles and Woods, 1964).

Priestnall (1972) showed that litter size could influence the time spent in licking by the mouse mother and found that pups reared in small litters received significantly more licking than pups in larger litters. Such mothers would also spend more time in the nest, nursing.

Licking of the pups in association with other actions may be moderated by several factors such as pup ultrasonic calls and olfactory cues (Noirot, 1969a).

Lactating females, males and even 'naive' mice are extremely responsive, when presented with very young pups. Noirot (1969b) showed that of such responses, retrieving always occurred first, and the lactation position (when displayed) usually occurred last. Licking was most likely to occur after retrieving, except when carrying the pup seemed difficult so that the pup was frequently dropped. In such cases, nest building usually occurred second. Noirot suggests that a pup outside of the nest emits ultrasonic calls which guide the parent to the lost pup and stimulate retrieving and nest building. Rough handling may cause the pup to change its call to the distress form and the adult mouse may drop the pup and return to the nest. Once dropped, distress calls of the former 'isolation' type are given by the pup and the adult responds. When retrieving is achieved, the pup usually stops calling. By the time the eyes have opened (day 14) the rate of ultrasonic calling is greatly reduced and the incidence of retrieval by the mother diminishes.

Reflex Development

Postnatal development involves maturation of several systems. In the case of the mouse, observations on newborn young have shown that mice progress through a pattern of reflex muscle actions and normal development can be ascertained according to several tests of reflex response (Fox, 1965). Such tests include: righting reflex, crossed extensor reflex and grasp reflex. Bolles and Wood (1964) included a study of reflex actions which occur at a later stage in development of

albino rat, such as the 'freezing reflex', which did not appear until day 23 when it was elicited by a sudden noise.

Nutrition influences development (MacDowell et al., 1930) and has considerable effect on the neuro-ontogeny of the mouse. Fox (1965) studied several mouse runts and showed that maturation of various systems was impaired. The eyes opened much later 15 - 17 days of age and weak body righting and locomotion was observed. Primitive responses such as rooting and pivoting persisted and weak bar-holding and grasp responses at 18 days of age indicated that muscular weakness was coupled with delayed ontogenesis of adult behaviour.

Altman and Sudarshan (1975) made a detailed study of the postnatal development of the laboratory rat and correlated the stages in motor development with maturation of sensory, motor and neural mechanisms which mediate such development; their aim was to establish criteria against which to assess retardation produced by various environmental agents. It has been found that exposure to low level x-rays resulted in the late development of locomotor skills. Such retardation was also seen following severe malnutrition during the suckling period, a treatment that affects cerebellar development.

Behavioural Aspects of Lactation

Suckling

Suckling is the defining behavioural characteristic of mammalia, yet little is known about either the factors that control newborn suckling or the means by which these controls recede during the course of ontogeny. (Teicher and Blass, 1976).

The mammary gland is an arrangement of the circulatory system and specialised tissues, enabling the synthesis and secretion of milk by certain epithelial cells. The milk of the rat contains a much higher percentage of fat than both human and bovine milk (10%), although considerably less lactose (2.6%). Other principal components are milk salts, and complex organic components including water-soluble vitamins at trace level. A variety of carbohydrates occur in the milks of various mammals, usually at levels far below that of lactose. Much of the carbohydrate is contained in glycoproteins and glycolipids and in the rat, water forms 79% of the milk.

Development of the lobuloalveolar system of the mammary gland depends on oestrogen and progesterone from the ovarian follicle, corpus luteum and placenta and the influence of hormones such as insulin and prolactin. The actual release of milk (milk ejection) is achieved by contraction of myoepithelial cells under the influence of oxytocin released in response to the sucking stimulus of the young.

Milk ejection in some mammals may be possible to 'condition': thus it has been suggested that for the cow the noise of buckets and even the sight of the milking-shed may act as an important conditioned stimuli for milk ejection (Kiley-Worthington, 1977). In mice and rats, adoption of the nursing position by the female is a common reaction in response to olfactory, or sound stimuli of young pups. The actual flow of milk thereafter is then mainly controlled by the suckling actions of the young.

Unlike newborns of other species which are led to or placed on the nipple, newborn rats and mice essentially locate and suck the teat without maternal assistance, even from the beginning. Also in contrast

with others, such infants do not suck objects other than the teat. Thus sucking in the blind and deaf newborn rat is controlled by a narrow spectrum of stimuli - olfactory, thermal and tactile - that define the mother and more importantly, her mammary area. (Teicher and Blass, 1976).

Kovach and Kling (1967) were able to show in the kitten that several factors controlled the ability to suck. Destruction of the olfactory bulbs rendered kittens unable to initiate sucking on the mother but did not interfere with the learned response of sucking on an artificial nipple. In further experiments 36 kittens were removed from their mothers and put into isolation and force-fed by stomach tube. They were returned to the mother after separation for various periods. Mortality was very high, with 69% of the kittens dying before reaching the set age for return to the mother. In kittens who survived, varying degrees of sucking difficulties were found which corresponded with length of time away from the mother. Between 6 - 19 days of age, kittens were able to establish sucking with increasing difficulty but after 23 days of age they were unable to either find the nipple or initiate sucking.

Kali et al ., (1971) found that newborn rat pups could be separated from the mother for up to 12 hours without deleterious effect on their subsequent development, although primiparous rats were reluctant to suckle their day old pups after a longer (16 hour) separation.

Teicher and Blass (1976) carried out extensive experiments on rat pups and found that sucking could be prevented if an olfactory chemical cue, present on the mother's nipples was washed away. Pups of 4 - 5 days of age were unable to find or attach to nipples, even when held

directly in contact with them. Replacement of the chemical cue allowed normal sucking to be reinstigated. They suggested that the cue was derived from rat-pup saliva; a variety of control substances including saline, water, liquid diet and mother's urine failed to influence pup attachment and suckling ability.

Ultrasounds

Isolation Calls

The young of a wide variety of rodents emit ultrasonic calls when they are removed from the nest. The ultrasounds produced by infant mouse (Mus musculus) were first studied in detail by Noirot (1966) and by Noirot and Pye (1969), who removed young albino mouse pups from their nest and isolated them in a dish or on the bench top for 5 minutes daily from birth onwards. Such calls in 'isolation conditions' were mainly 10-140 ms in duration at frequencies between 45 kHz and 88 kHz.

The frequency generally changed slowly over the length of the pulse but sometimes small instantaneous jumps in frequency occurred. The rate of calling increased markedly on the fourth day after birth and then decreased to zero on the day on which the eyes of the young opened, i.e. about the thirteenth day. These workers also noted that more ultrasonic calls were detected when the young were being retrieved by their mother than when they were isolated.

Such studies confirm the earlier observations of Noirot that such maternal responses as retrieving, nest building, licking and covering the young in a nursing position were not mainly dependent on the sex or hormonal state of the adults. Cues coming from the young themselves were also of great importance. Similarly, work by Zippelius and Schleidt showed that lactating female mice would retrieve live pups from outside

the nest but would ignore dead or anaesthetised young that were unable to elicit ultrasonic calls.

Distress Calls

Following the studies by Noirot, who proposed that ultrasonic calls in baby mice served the dual function of eliciting retrieval responses and inhibiting maternal aggressive behaviour, Sewell (1968) looked at distress calls in several strains of mice. She found little difference in frequency pattern, and noted that sound emissions did not necessarily cease when the eyes of the young opened. Both the intensity of the calls and the time of cessation depended on how the young were treated. Calls could be obtained up to 20 days of age in EN mice and 39 days in C₃H mice, if the mice were lightly traumatised by pinching or rolling.

Such 'handling' calls were always louder than the 'isolation' calls and Sewell suggested that these two different levels of intensity may reflect the two different functions of the calls previously suggested by Noirot. The less intense 'isolation' calls may be motivated by cold and hunger and initiate the retrieving response of the mother, whereas the louder calls produced on handling may inhibit the mother's aggression and so persist to a greater age, until the offspring can fend for themselves.

Effects of Cold Stress

Studies by Hart and King (1966) on the North American deer mouse (Peromyscus maniculatus) suggest that the rates of calling may be related to the stage of homeothermic development. P.M. bairdii ceased to produce calls between 10 and 12 days of age and P.M. gracilis between 14 and 16 days. Hart and King suggested that since this is

probably about the time when the young begin to regulate their own temperature effectively. The calls induced by cold stress may guide the mother to her young and so restore the pups to their primary source of heat. Once the pups are able to thermoregulate physiologically, cold stress apparently no longer elicits distress calls.

There are three stages in the development of homiothermy in baby mice as described by Okon (1970). For 5 - 6 days after birth the pups are poikilothermic, adopt a temperature similar to that of their surroundings, and can tolerate hypothermia. At day 6 - 7 until day 14 the pups begin to regulate their own temperature and are then much less able to tolerate hypothermia. Okon found that rates of ultrasound calling at this time increased from 4 calls to 32 per minute and were of greater intensity than the calls of younger pups. Animals maintained at a very low ambient temperature ($2 - 3^{\circ}\text{C}$) produced much more intense calls at day 10 - 11, than similar animals at an ambient temperature of 33°C , many of whom did not call at all.

The concluding homiothermic phase lasted from day 14 until day 19 - 20. Such animals when isolated ceased to emit ultrasounds at different ages depending on the ambient temperature; animals at room temperature ceased calling at day 13 but animals kept cold ($2 - 3^{\circ}\text{C}$) continued to produce ultrasonic calls until the age of 20 days when full homiothermy was achieved. Conclusive evidence of effect of cold stress was obtained by Okon in experiments on pups aged 7 - 14 days. Pups which had ceased calling at 33°C were left for a further 10 - 15 minutes at this temperature and then their ambient temperature was allowed to fall. The pups began to produce prolonged ultrasound calls below 30°C . On raising the temperature again, calling ceased.

Exploratory Behaviour

Tests of 'emotionality', 'fearlessness' or 'exploratory behaviour' have been widely used in experimental studies of rodent behaviour, and have been reviewed by Archer (1973)

Such tests include the use of several different types of 'novel environment', in which the animal is simply placed in an unfamiliar situation. In addition a number of more arbitrary and bizarre tests have been used in some cases; some involved submerging the animal partly or totally in water (underwater swimming, water maze, water-wading, defecation test) another measures the latency to descend from a pole by means of a ladder.

Such tests have been used to assess the effect of genetic differences, prenatal treatments, neonatal stimulation, postweaning housing conditions, sex differences and other variables on 'emotionality' or exploratory behaviour. Most commonly, this type of research involves the open field test and measures ambulation and defecation.

By interbreeding only the extremes in each generation, a number of investigators have produced strains of rat which are widely separated in emotionality and activity, suggesting that these traits are definitely dependent upon heredity and genetic makeup (Thompson, 1953). Thompson examined 15 different mouse strains and found significant differences in food-drive, emotionality, and exploratory behaviour. He found no definite relationship between the behavioural traits studied and coat-colour or other morphological characteristics. However, although rearing techniques and environment were quite standard it was suggested that slight differences in handling, maternal care, position of home-cage in relation to light and other such factors may have

introduced some variability. Since some of the most highly inbred stocks showed as much variability as less inbred ones, it is doubtful if further inbreeding would result in appreciably greater with in-strain homogeneity.

Thompson suggested that major differences in activity could be due to variation in early environment of the test animals. Rearing the subject in a 'free environment', providing a wide variety of stimulation from a very early age, could result in a heightened threshold of reactivity.

Denenberg et al. (1963) described an experiment where inbred strains were compared on a series of behavioural tests, including activity in an open field. The mutants, 'pintail', 'hairless', 'pale ear' and 'short ear' were tested. Neither 'pale ear' nor 'short ear' animals differed from their controls on any test but 'hairless' mice were found to be significantly less active than controls in the open field test.

In another study, animals presenting with a 'pink eye dilution' and 'jerker' characteristics were compared with normal controls. Homozygous pink-eye animals made a particular type of visual orientation response and a paw-lifting response less often than controls. Jerker mice were found to be affected in exploratory activity more than in fighting, feeding and sexual behaviour. Such studies demonstrate that even relatively small changes in chromosomal material may have demonstrable effects on behaviour.

Some aspects of physiological effects on behaviour should also be noted. It has been found that thyroid activity in certain strains of mice correlate with locomotor activity levels. Similarly it has been suggested that brain serotonin levels show an inverse relationship to 'emotionality', among several strains of mice. McGill (1962) has studied in detail the male sexual behaviour of three inbred strains of mice. C57BL mating pattern was found to be rapid with quick intromission and ejaculation, whereas BALB/c males required a much longer time with ejaculation taking up to one hour to complete. Such behaviour must lead to unfavourable reproductive prospects in a natural situation.

(b) GROWTH AND DEVELOPMENT OF MICE

There has been a considerable amount of information published about experiments involving growth processes in rodents, particularly the rat, by workers in such fields as nutrition and endocrinology. The criterion of growth chosen by most workers has been that of body weight; the reports of measures of growth other than weight are far less numerous and generally concern changes in skeletal length (Hughes, 1968).

The techniques for the measurement of physical dimensions in man and animals are well established and many dimensions may be studied, e.g. skeletal ones such as bi-iliac diameter, bi-acromial diameter or femoral condyle width, or others such as limb circumference or sub-cutaneous fat thickness. In addition to anthropometric techniques, photogrammetry is often used enabling further measurements as well as information about body shape and maturity, to be obtained from the resulting photographs.

Body Weight

Body weight has been the criterion chosen by most workers as a measure of growth in rats and mice but comparisons between studies are complicated by differing nutritional conditions, litter size, inability of the mother to provide sufficient milk, etc.

MacDowell et al. (1930), reviewed the available literature concerning growth curves (body weight) for suckling mice and showed that, considering the great range in material, methods and laboratories, the curves were fairly consistent. He showed that body weight increase could be greatly stimulated by reducing the number of suckling young.

Scudder et al. (1967) examined the body weight curves for 6 strains of mice and showed that growth curves show two breaks. The first probably occurs at the onset of decline of the mother's milk supply, (at day 7) and the second correlates with the opening of the eyes, when the mice begin the transition from milk to solid food.

In studying rat pups from birth until 16 days of age, Schultze (1954), showed that with small litters, e.g. 6 pups, the mother was not induced to maximum milk production. By increasing the litter size there was a progressive decrease in the mean weight increase of each pup but a marked increase in the total weight gain of the litter, reflecting greater milk production of the mother. In such cases it was possible to maintain or increase the weight of suckling pups numbering 11 or more if suitable maternal diets were made available.

To achieve ample milk production, enabling normal growth of newborn mice, the mother must obtain sufficient nutrition and this is normally associated with increased food consumption, which may rise by 100% at day 5 post-partum (Smart and Silence, 1977)

Skeletal Size

The data on skeletal dimensions are far less numerous than those available for weight. The body or tail lengths of animals have been included in some published studies such as those of Moment (1933), who measured body lengths on dead animals. Other studies have included the measurement of body lengths and limb lengths on anaesthetised animals, and the measurement of naso-anal length and skull length.

In the mouse, Dice and Bradley (1942), measured the length from snout to anus and anus to tail tip using a metric rule. Similar

studies have been done on the house mouse, where isolated bones were also measured using vernier callipers or micrometer. Cross-sectional study of the skeletal components has involved killing the animal and studying the dry bones or alizarin preparations. The use of radiographic techniques to follow the growth in rats has been widely used and has been reviewed by Hughes (1968).

The concept of 'shape age' utilises the fact that there is a changing relationship of body proportions with increasing age. This criterion has been used by a number of workers including Dickerson and Widdowson (1960) and Widdowson and McGance (1960) who used the ratios tail length: total length and femur length: femur breadth to assess the effects of undernutrition on rats.

Dental age in human studies utilizes the eruption, non-eruption, or the achievement of a particular shape of a tooth in order to assign a maturity rating to it. In more detailed studies the degree of eruption and the degree of root or crown development is determined radiographically. In sub-human primates dental appearance has been used to assign ages to animals and in rats and mice several investigators have used dental age for assessing physiological maturity.

The Normal Growth Pattern of Mice

The growth of a mouse may be divided conveniently into four periods: 1) prenatal, 2) birth - D30, 3) D31 - D60, 4) D61 - senescence (Butler and Metrakos, 1948). Growth during the prenatal period is influenced markedly by the site of implantation of the fertilized ovum and by the amount of blood supply received by the growing embryo.

It has been shown that the birth weight varies inversely with the litter size, but the relationship is not constant for all strains. The D0 - D30 period is not a homogeneous one, but is marked by two distinct lag phases, according to Butler and Metrakos. The first lag phase occurs soon after birth and lasts for about 2 days. The second lag phase occurs at about D14.

The subsequent growth rate reaches a plateau and the rate of growth becomes quite uniform for the rest of the life span. A small period of retardation, however may occur at around D44, corresponding with the time of puberty.

In the early stages of postnatal growth two of the most important factors influencing growth are litter size and quantity and quality of the mother's milk. MacDowell et al. found that the differences of weaning weights between mice raised in small and large litters persist for a long time, whereas studies with rats showed that animals from similar groups returned to normal size by seven weeks of age.

Work on the hairless mouse (hr/hr) with normal thymus (Stanier, 1977) showed that neither growth rate nor the distribution of fat within the body of the growing mouse could be influenced by rearing the animals at different environmental temperatures, (i.e. 22°C or 33°C). Rearing at the higher temperature however, both decreased food intake and also promoted deposition of more fat within the body.

As it is often accepted that nutrition is the limiting factor affecting growth, workers on size inheritance of mice provide their animals with a high level of nutrition, while keeping the rest of the environment as uniform as possible. The one thing that is difficult to standardise is the quantity and quality of the mother's milk.

Nutrition

Less is known about the nutritional requirements of the mouse than of the rat, probably because it has been employed less extensively in nutritional research. Several investigators suggest that mice resemble rats in their nutritional needs, since there is some evidence indicating that certain diets permit equally satisfactory performance with both species. It has been recognised however that there are differences in requirements for nutrients between strains and between individuals within a strain (Lane-Petter and Porter, 1963).

If a diet provides adequate amounts of vitamins, minerals, sources of energy and other factors, the growth rate of young animals will be related to the level of protein. Increasing the protein content will usually elicit some further improvement in growth rate, so that fixing a recommended level of protein is to a large extent a compromise between cost and the benefits of improved growth and breeding performance. Estimates of the requirements for reproduction (Nelson and Evans, 1961) suggest that when expressed as a proportion of the diet, the need for protein during both pregnancy and lactation does not differ significantly from that for growth.

The presence in a diet of sufficient vitamins is essential for normal reproduction and growth. Vitamin A has been shown to be essential for the mouse at the level of about 1 - 2 IU per day. Deficiency symptoms include tremors, rough coat, poor growth, abortion and sterility in males. In the case of Vitamin D an absence may result in a rickets-type condition. A deficiency of Vitamin E may be less serious, although muscular dystrophy has occurred in Vitamin E deficient rats. It is generally accepted that Vitamin K is synthesised by micro-organisms of the alimentary tract in sufficient quantity to cover the needs of rats and mice that are maintained in

conventional conditions. A haemorrhagic condition of rats maintained in a specific pathogen free (SPF) colony has been reported by Gaunt and Lane-Petter (1976), who recommended the addition of at least 1.5 ppm vitamin K (as menaphthone) to the diet.

The rat and the mouse supplement their requirements of the Vitamin B complex through coprophagy, by which they obtain vitamins synthesised by micro-organisms in the lower part of the gastrointestinal tract. Thus, as for Vitamin K, a deficiency may occur in SPF housed animals. Even in conventional conditions, intensively-bred animals may be deficient, as precise requirements have not been defined. Excessive amounts of Vitamin A or D are toxic to animals but many times the physiological level can be given without serious harmful effects.

Other vitamins like thiamine, biotin, B₆ & B₁₂ are also required for normal growth and development of mice. Morris and Lippincott (1941) showed that lack of pantothenic acid in growing mice caused loss in weight, denuding of hair and dermatosis.

Quantitative data concerning the mineral content of rodent diets is limited but the importance of calcium, zinc, iron and potassium has been described. Iron may be important for both growth and reproduction; anemia may develop in iron-deficient young mice and birth weights and litter size may be lower than normal. Zinc has been proved essential in mouse nutrition and the potassium requirements of the growing mouse has been found to be 0.2% of the diet. Mice fed highly purified diets, deficient only in potassium died within one week and exhibited outward signs of inanition; 'Lustreless' eyes and haircoat, dry scaly tail and general emaciation were observed in connection with severe deficiency. Partial deficiencies resulted in poor growth and lack of 'bloom'.

Other important nutritional factors include energy needs, essential fatty acids and water. Rats and mice are customarily offered their diets ad libitum and the amount consumed will depend on each individual's requirements for energy. Since this will be influenced by such factors as environmental temperature and physical activity, and will also vary with physical performance and rate of growth, the level of intake will vary considerably. The main source of energy in diets for rats and mice is carbohydrate.

Possibly the only particular importance of fat in mouse diet is as a source of essential fatty acids and to promote the absorption of the fat soluble vitamins, although there is some evidence that lactating female mice may exhibit a specific hunger for fats. It is not clear whether this reflects a demand for energy or for specific nutrients.

The ad lib food intake of a growing mouse appears to be only about 1.25 times the maintenance intake required to keep it at a constant weight. Stanier (1976) found that when hairless mice were fed at 70-75% of their ad lib they failed to thrive, lost weight and died.

Mice should be provided with a continuous supply of water; mice fed dry rations and housed in temperatures of 75-80°F may perish if deprived of water for a day. There is little evidence to suggest that there are basic differences in energy metabolism between mice and other animals and water requirements may be proportional to energy intake, although Smart and Dobbing (1977) suggest that adult rats which have been undernourished in early life display increased thirst.

Genetics

The morphological, developmental and behavioural characteristics of individual mouse strains vary greatly. Thus the rate of weight gain, body length, colour of hair, ability to move through a maze or run in an activity wheel, the incidence of particular tumours or degree of aggression are just a few of the characteristics which have been shown to differ between strains and within different breeding regimes for the same strain.

The concept of 'genetically defined' strains is an important one. Inbred strains are the result of at least 20 generations of brother x sister mating. During the last 10 years many inbred mouse strains have been established but such colonies may suffer from certain disadvantages. Inbreeding depression, which is the reverse of hybrid vigour, affects the level of many aspects of reproductive performance, (and many of its components) and may result in the developing inbred strain becoming extinct before it reaches its sixth or seventh generation (Festing & Atwood, 1970).

Genetically undefined stocks of 'outbred' or 'random bred' mice, consisting of a population of animals mating at random and not subjected to selection, have been widely used. Such strains usually display good reproduction and produce large litters with high weaning rates.

The spontaneous natural mutation rate in mice has been estimated as ranging between 1-30 per 10^6 mouse reproductions and experience has shown that the occurrence of mutations must be considered to be an important factor in the maintenance of an inbred strain (Festing and Atwood, 1970). Where a mutation is visible it can be eliminated from

the strain with high probability, even though the mutation may be recessive. However, it is probable that many mutations occur which are invisible but which can influence characters such as fertility, litter size, etc.

Many of the mutations have a pathological effect such as paralysis, obesity, anaemia, or in the case of the 'nude mouse', absence of hair coupled with absence of the thymus gland. Such animals can be considered 'models' for the human disease and used as 'tools' in medical research. There are currently more than 60 such mutant strains of mouse maintained either as outbred or inbred colonies (see table 2.)

Human Disease	Animal Model	Human Disease	Animal Model
Achondroplastic dwarfism	<i>cn/cn</i> achondroplastic mouse	Muscular dystrophy	<i>dy/dy</i> , <i>dy²/dy²</i> dystrophic mouse
Adrenocortical lipid depletion	<i>ald/ald</i> adrenal depleted mouse	Myelin defects, CNS	<i>jp/Y</i> , <i>jp^{msd}/Y</i> , <i>jimpy</i> ; <i>qk/qk</i> , quaking, mice
Anemia with leukopenia	<i>an/an</i> Hertwig's anemia	Myodystrophy	<i>myd/myd</i> mouse
Ataxia	<i>ax/ax</i> ataxic mouse	Nieman-Pick disease	<i>fm/fm</i> , foam-cell reticulosis in mice
Autoimmune hemolytic anemia	NZB/B1 inbred mouse	Obesity, hereditary	<i>ob/ob</i> , <i>A^y/-</i> , <i>fat/fat</i> , three kinds of fat mice
Cataract	<i>Cat/+</i> dominant cataract mouse	Osteopetrosis	Osteopetrotic rabbit and mouse
Central core disease (loss of Purkinje and granule cells)	<i>tg/tg</i> , <i>tg^h/tg^h</i> tottering mouse	Osteosclerosis	<i>oc/oc</i> mouse
Chediak-Higashi syndrome	<i>bg/bg</i> beige mouse	Otolith defect	<i>pa/pa</i> pallid mouse
Chondrodysplasia	<i>cho/cho</i> chondrodysplastic mouse	Phosphorylase-B-kinase deficiency	<i>pdk/Y</i> sex-linked kinase deficiency in mouse
Coloboma	<i>Cm/+</i> mouse	Pituitary dwarfism	<i>dw/dw</i> , <i>df/df</i> , two kinds of dwarf mice
Congenital glaucoma	<i>bu/bu</i> buphthalmic rabbit	Prolinemia	<i>pro/pro</i> prolinemic, prolinuric mouse with proline-oxidase deficiency
Copper-transport defect	<i>Mo</i> , <i>Mo^{br}</i> , <i>Mo^{to}</i> , sex-linked mottled mouse	Reticulum cell sarcoma	Old C57BL/6 mouse
Cribiform degeneration	<i>cri/cri</i> mouse	Retinal degeneration	<i>rd/rd</i> mouse
Diabetes	<i>db/db</i> , <i>db^{ad}/db^{ad}</i> diabetic mouse	Siderocytic anemia (transitory)	<i>sfl/sfl</i> , flexed mouse
Deafness	<i>dn/dn</i> mouse; in other mutants associated with inner ear defect	Spherocytic anemia	<i>sph/sph</i> mouse
Dystonia muscularum	<i>dt/dt</i> mouse	Splenless	<i>Dh/+</i> dominant hemimelic mouse
Iron-transport defect	<i>Sla/Y</i> sex-linked mouse anemia with defect in iron transport	Slipped discs	<i>Pr/+</i> pintail mouse
Hemolytic anemia	<i>ja/ja</i> , <i>Nb/nb</i> , <i>Ha/ha</i> , and <i>sph/sph</i> ; four distinct mouse hemolytic anemias	Tay-Sachs disease	<i>ot/ot</i> , oscillator mouse
Hepatoma	<i>dan/dan</i> hemolytic dog anemia, with Na ⁺ defect	Testicular feminization	<i>tfm/Y</i> sex-linked testicular feminization in mouse
Hodgkin's disease	High incidence in (DBA/2 X CE/J) _{F1} , and in C3H/HeJ males	Thymusless	<i>nu/nu</i> , nude mouse lacking thymus
Hydrocephalus	SJL/J mouse	Vitamin-D resistant rickets	<i>Hyp/Y</i> sex-linked dominant with hypophosphatemia and <i>Hyp/+</i> shortened, weak long bones, in mice
Hydronephrosis	<i>ch/ch</i> , <i>hy-3/hy-3</i> ; two kinds of hydrocephalic mice	Microcytic anemia	<i>mk/mk</i> mouse
Hypoplastic refractory anemia	<i>se/se</i> short-eared mouse	Microphthalmia	<i>Mt^{wh}</i> , <i>mi</i> , other <i>mi</i> alleles (<i>mt^{rw}</i> , <i>mt^{sp}</i> , etc.) with effects on eye size and pigment
Ichthyosis	<i>w/w^u</i> , <i>S1/S1^d</i> , and <i>an/an</i> mice with macrocytic anemia	Multiple myeloma	Plasma-cell tumors in BALB/c mice
Immune response	<i>ic/ic</i> mouse	Muscular dysgenesis	<i>mdg/mdg</i> mouse lethal with abnormal nonfunctioning muscle
Lack of pituitary growth hormone	<i>Ir-1</i> locus		
Lipofuscin granule deposit	<i>lit/lit</i> , little; probably growth hormone defect		
Lymphocytic leukemia	Old C57BL/10 mice, plus others		
Genes related to leukemia viruses	Inbred strains: AKR/J, C58/J		
Mammary adenocarcinoma	Single genes: <i>S1/S1^d</i> , <i>hr/hr</i>		
Megacolon	<i>MLV-1</i> , mouse leukemia virus		
	<i>FV-1</i> , <i>FV-2</i> (Friend's virus)		
	<i>Gv-1</i> , <i>Gv-2</i> (Gross virus)		
	C3H/HeJ mouse, and especially C3H/HeB-A ^{vy}		
	<i>ls/ls</i> , <i>s^h/s^h</i> mice (two types of spotting-with-lethality)		

Table 2 Examples of Genetically defined Animal models of human disease (from the Institute of Laboratory Animal Resources ILAR NEWS 19, No. 2 January 1976. Publish by Division of Biological Sciences, National Research Council, Washington D C. U.S.A.)

(C) THE NUDE MOUSEHistory

This mutant mouse was first described in detail by Flanagan (1966). Dr. N.R. Grist of the virus laboratory, Ruchill Hospital, Glasgow, observed a hairless mouse within a closed but not deliberately inbred colony of albino mice. This animal together with two phenotypically normal mice, male and female, from the same litter, were sent to the Institute of Animal Genetics in Edinburgh.

The normal coated mice were supposedly heterozygous for a recessive gene which was responsible for the mutation - and so it has proved. Flanagan (1966) after a series of reproductive studies showed that a single autosomal recessive gene was responsible and he gave the name "nude" to the animal, presumably to differentiate between this mutation and the "hairless" mouse that had been discovered previously.

Flanagan studied intercross matings of heterozygous $nu/+$ mice comprising 122 matings with a total progeny of 5,239 mice, 1,349 (25%) of which were nude. Observations on homozygous nude mice showed that they were often infertile, particularly females, but from four matings of male nu/nu with female $nu/+$, a total of 30 young were obtained, 16 of which were heterozygous nu/nu as expected, confirming that the gene was autosomal recessive.

In his paper Flanagan noted that although there was no evidence of increased pre-natal mortality of nude mice, post-natal mortality reached 100% by 25 weeks. Death was usually preceded by rapid loss in weight and an apparent inability to compete with normal littermates for food. While many of the survivors at two weeks were weak some continued

to live for considerable periods. The mortality of normal mice was 6%, no greater than could be expected under standard conditions.

Failure to obtain viable adult mice prompted Flanagan to carry out detailed post-mortem examinations. All dead mice showed a severe liver disease; liver lobes were atrophied and covered with red scars. The heart, lungs and spleen appeared normal. Subsequently, all moribund or dead mice were examined for this defect. Table 3 summarises the frequency of liver disease at different ages, but it is interesting to note that only one animal showed the disease before 3 weeks of age. As Flanagan's own results show, (Table 4) a high percentage of nude mice died before 3 weeks of age and therefore causes other than infection or other disease must be responsible.

It was not until 1968 that Pantelouris explained the reason for the high mortality observed in weaned mice. Examination of a number of nude mice showed them to be deficient in thymus tissue, and blood tests displayed extreme leucopenia. Normal coated mice were found to have fully developed thymus glands and total white blood cell counts were similar or up to four times higher than nudes tested. Lacking in T-lymphocytes, nude mice were therefore unable to mount an adequate defence against pathogenic micro-organisms and disease conditions in general.

The frequency of liver disease among moribund or
dead nude mice

Age	No. of nude mice dissected	No. of nude mice with liver disease
Birth to 3 weeks	56	1
3 weeks to 6 weeks	31	16
6 weeks to 26 weeks	85	85

Table 3

The mean weights of nude and normal mice at birth,
3 weeks and 6 weeks of age

		Nude		Normal	
		♀♀	♂♂	♀♀	♂♂
Birth	No. of mice	120	138	288	338
	Mean wt. (g)	1.5	1.6	1.5	1.6
3 weeks	No. of mice	42	65	103	115
	Mean wt. (g)	6.0	6.6	10.1	10.3
6 weeks	No. of mice	10	18	23	27
	Mean wt. (g)	14.6	18.4	22.6	26.7

Table 4

Table 3 and 4

Mortality and Growth Results: Flanagan (1966)



Fig 1. Wasting Disease in an Adult Nude mouse, maintained in conventional conditions.

Appearance and Growth Rate

Nude mice can be identified at birth by the absence of whiskers (Flanagan, 1966) or by poorly developed crinkled whiskers (Rygaard and Friis, 1973). Young animals fail to grow a first coat. Occasional sparse hair growth may appear, showing cyclic changes. A similar cyclic growth of whiskers may occur, the crinkled appearance being a constant feature in younger mice.

Flanagan found that at weaning, nude mice were much smaller than hairy mice and were often in poor condition. He suggested that the nude gene exercised an appreciable influence on body growth; his experiments are summarised in Table 2. There was no difference in the birth weight of nude and normal mice but at 3 weeks the difference was considerable. The weight difference was similarly pronounced at 6 weeks of age; on a percentage basis nude female mice were only 64.6% of the weight of normal females and nude male mice were 68.9% of the weight of normal males. Similar results were found by other workers, and the difference in growth was maintained for both conventional and isolator-maintained colonies (Poiley et al., 1974).

Irrespective of genetic background, all nudes look very similar; most characteristic is the almost complete lack of body hair. Areas of sparse hair growth may occasionally be seen at various sites over the trunk, a phenomenon most marked in mice of C57BL background, least in those of C3H. The whiskers are invariably deformed or atrophic. The skin may be fairly thick and wrinkled, or very thin and smooth.

Skin pigmentation, varies distinctly with genetic background. The skin of BALB/c nudes is light reddish in colour and the eyes are albino. C3H nudes have uniformly light greyish skin and the eyes are darkly

pigmented. The skin of C57BL nudes varies from dark greyish to black. There is great variation within and between individuals. As observed zonal variation is related to the hair growth previously mentioned. Pigmentation is most marked in these areas.

Reports of the longevity of nude mice within different environments vary greatly. The observation of Flanagan (1966) that in conventional conditions the life span of nude mice is very short has been confirmed by several other workers, (Wortis et al., 1971; Sebesteny & Hill, 1974; Fogh & Giovanella, 1978). Under germ-free conditions however, the average life of homozygous nude mice is greatly increased, and may reach 9 - 12 months (Rygaard, 1973; Festing & King, 1974).

In a further study, reviewed by Gullino et al. (1976), selected strains of nonpathogenic bacteria, resistant to the antibiotics employed, were allowed to colonise the intestine of the nudes. This increased the effectiveness of the other precautions by competitively blocking the development of undesirable bacteria as well as improving the digestive function of these animals. It is claimed that under these conditions, the life expectancy of outbred Swiss-background nudes average 18 months, with many animals surviving two years.

Absence of the Thymus

In the mouse the thymus derives from the third pharyngeal pouch which is already recognizable in the 3 - 4 day old foetus. Development continues so that at birth (19 - 21 days) the thymus is fully descended and separated from the parathyroid. Rapid postnatal growth occurs at a rate dependent on the strain of mouse concerned. In newborn mice (body weight about 1.5 g), the thymus weighs 15 mg; approximately 1% of body weight. At 6 - 8 weeks thymus weight is

maximal approximately 60 mg, but then constitutes only 0.3% of body weight. At the age of puberty growth of the thymus is arrested, weight of the organ falls and there begins a progressive replacement of active thymic tissue by fatty connective tissue.

In their studies on the prenatal development of the nude mouse, Pantelouris and Hair (1970) demonstrated that the thymus rudiment of 14 day old nu/nu foetuses already differed from that of the nu/+ animals. The nudes are born with only a cystic rudiment of a thymus but have the normal precursors of antibody-secreting (B) cells and thymus-dependent (T) cells. In the absence of functional T-cell competence the nude mice have no graft rejection or graft vs. host response.

The possible role of endocrine factors in the thymus and their control by hormones of the adenohypophysis or its target glands has been investigated extensively in animal models. Pierpaoli and Sorkin (1972) reported a study of the relationship between the thymus, the adrenal cortex and the thyroid gland, with reference to the nude mouse. They suggested that the progressive symptoms of rapid diminution of body weight, loss of subcutaneous fat, dehydration, with hypothermy and hypotony before death in mice affected by 'wasting' disease may be the result of endocrine disturbances. Experimentally neonatally thymectomised normal mice developed similar symptoms at 25 days of age. Examination of their adrenal glands and glands from 'wasted' nude mice showed that the adrenal cortex was shrunken, the zone reticularis was absent and replaced by cuboidal cells. When newborn nudes had a nu/+ thymus transplant the adrenal glands developed normally.

Ohsawa et al. (1974) carried out intensive investigations into the pituitary, adrenal, thyroidal and gonadal functions of nude mice, before and after treatment with calf thymus extracts. They concluded that nude mice in the SPF state with known genetic and microbiological background had intact endocrine functions when compared with BALB/c controls. The injection of calf thymus extract had no appreciable influence on such functions. Their growth curves showed that until 3 weeks of age, i.e. the suckling periods, the body weights of nude mice were lower than those of controls. However, after weaning the growth of nude mice approached control values, which suggests that the functions of the growth hormone in nude mice are not significantly disturbed.

Husbandry

Artz (1972) found that approximately 33% of nude males that reached sexual maturity (40 days) were fertile. Such animals were caged with 4 - 5 females of an inbred strain, (Balb/c). F1 nu/+ mice were then mated inter se in cages of 1 ♂ + 1 ♀, without subsequent isolation of pregnant females, to take advantage of post-partum oestrus.

The F2 litters then contained 25% nu/nu pups. Artz found that both F1 and F2 mice were extremely vigorous and fertile and that the females made excellent mothers. The normal litter size was 8 - 12 and each litter contained 1 - 4 nudes. He observed that the nude pups were runt, "because of their athymia" and that they experienced great difficulty in competing with normal litter-mates for food.

Therefore two main husbandry techniques were employed.

- (1) Ten - Twelve days after birth all normal mice were culled leaving only nudes in the nest. However, females would only rear successfully litters of more than three pups.
- (2) When several litters had been delivered on the same day, all nudes were transferred to a single foster mother. Fifty percent of nudes achieved weaning age.

Giovanella et al. (1973) initially experimented with animals carrying double markers in combination with the nude gene. The male carried the Rex phenotype (curled hair) and the female, the trembler phenotype (generalised tremor, spastic paralysis and convulsions when stimulated). Of two pairs, one proved heterozygous for the nude gene and their colony was developed from the progeny of this pair. Male 'Rexes' which carried the nu gene (i.e. at least one nude in their offspring) were mated with C57BL/6 or Swiss mice. After a few trials, only Swiss mice were used because of their larger litters.

The later breeding schemes used healthy pathogen-free homozygous nude males crossed with females heterozygous for the nu gene. Fifteen nudes were obtained by directly mating male and female nudes but they observed that in general nude females were poor nurses and frequently cannibals.

Serious large scale breeding reports (Poiley et al., 1974; Holmes and Mason, 1974 and Festing and King, 1974) all stress the need for specific-pathogen-free (SPF) or barrier maintenance conditions as a pre-requisite for successful breeding and longevity. Using either isolators or barrier rooms, Poiley et al. (1974) were able to keep

95% of mice alive for more than 6 months. Festing and King (1974) reported that their mice kept in strict SPF conditions (Medical Research Council 4-star) on average lived longer than 9 months.

Giovanella et al. (1973) isolated their SPF mice in sterile plastic containers, covered with non-woven, spun polyester filter covers that allowed the passage of air but not dust or bacteria. Tissue paper was used for bedding. Autoclaved water bottles were filled with tap water containing 4 g/L neomycin, 4 g/l bacitracin and 1 ml/l multivitamin. The solution was sterilised by millipore filtration, (0.22u filter). Acidification of drinking water with hydrochloric acid to pH 2.5 - 2.8 has been described as effective in controlling contamination rates, particularly in regard to *Pseudomonas* species (McPherson 1963), although it has been suggested that the combination of acidification and chlorination is deleterious to the general health of laboratory mice.

Gullino et al. (1976) state that any commercially produced pelleted mouse diet is suitable for nude mice. As many commercial diets are contaminated with Enterobacteria (Williams and Habermann, 1962), autoclaving for 45 minutes at 120°C is necessary. If as a result excessive loss of vitamins occurs a polyvitamin concentrate may be added to the drinking water, although a daily change of water bottles is then recommended. They also suggested that for nursing mothers on a normal diet a supplement of whole-wheat bread and either condensed or reconstituted milk powder may be effective in preventing cannibalism.

It has generally been accepted that nude mice require a warmer ambient temperature than normal mice and a range of 26° - 28°C has been

recommended (Rygaard, 1973). Nudes housed in a continuous laminar-air-flow environment need a temperature of at least 28°C to compensate for their more rapid loss of body heat.

Summary of Literature in relation to the Nude Mouse

In discussing his original paper on Nude mice, Flanagan (1966) suggested that the hairlessness was caused by abnormal keratinization of hair in the follicles, low fertility was due to non-mobile sperm, small ovaries and low egg counts, and that the cause of the 'liver disease' (and death) had not been determined but the defect had been traced to its initial stage, i.e. necrosis of small areas of parenchymal tissue at various points throughout the liver.

In view of his extensive and comprehensive breeding, pathological and genetical studies it is surprising that he did not observe that his nude mice were lacking a thymus gland, as confirmed two years later by Pantelouris (1969).

Flanagan found that during the first week of life many pups died of 'general body weakness'. Festing (1974) using SPF techniques found that longevity of nudes could be extended to two years but agreed that there was some indication of increased mortality of homozygotes, prior to weaning. Fogh and Giovanella (1978) in their treatise on nude mice added little more than 'The short life span of mutants is not unusual, since many of these mutations are deleterious'.

With the realisation that animals were dying from simple infections due to their (un)natural immunosuppression it was a logical step to then initiate breeding nuclei from SPF animals and maintain resulting offspring in carefully controlled environments. However, higher than normal death rates for nudes continued to be found.

Flanagan's work also suggested that 'the nude gene exercises an appreciable influence on body growth'. Although there was no difference

in the birth weights of nude and normal mice, by three weeks of age the difference was considerable. Authors using animals from a variety of genetic backgrounds, were later in agreement that this was the case (Poiley et al., 1974; Festing and King 1974; Fogh and Giovanella 1978).

Discounting the influences of infectious agents (obviated by good maintenance regimes), a number of other causes of early perinatal death in suckling pups, must be considered.

Aden et al. (1972) reported that nude mice were continuously kept with normal littermates to 'delay the onset of wasting disease'. Other authors have also suggested that in keeping nude offspring with some hairy sibs, nudes tend to survive, (Giovanella and Stehlin, 1973). Hetherington and Hegan (1975) however, suggest that it is advisable to remove phenotypically normal pups from the litters as these grow faster than their nude littermates.

In keeping the litters intact, a high level of pup stimulation is maintained on the mother, eliciting a normal maternal response and nursing behaviour. Removal of too many pups at the wrong time post-partum could adversely affect this relationship.

However, if the normal hairy pups tend to grow at a greater rate than nude pups, then maintaining a large number of pups in the nest could result in less milk being available to satisfy the needs of some nude pups.

The effects of removal of hairy mice from mixed litters have not been studied in detail and little indication has hitherto been given of effects related to age of pups removal, although Artz (1972) did

suggest that mothers would nurse litters of more than three nude pups after removal of hairy sibs; 50% of nudes only, however, achieved weaning age.

Limited observations by some authors have suggested that nude mothers lack normal maternal abilities, resulting in early death of nude pups. No work has been undertaken to describe the degree of maternal behaviour in heterozygous hairy mothers, although all reports indicate that such mothers are successful in rearing mixed litters.

Authors have readily cited the physical differences between nude and hairy pups in descriptive terms but no measures have been taken of other parameters, such as reflex development or locomotion etc.

Therefore, much behavioural and developmental data relating to the nude mouse is lacking, although in the past 12 years this mutant has been the subject of probably more immunological and transplantation studies than any other experimental animal.

CHAPTER 3

NUDE MOUSE HUSBANDRY: TECHNIQUES USED IN THIS STUDY

Introduction

The colony used for the production of nude mice used in this study was established in 1973, using a nucleus of animals obtained from the MRC Animal Centre, Carshalton, Surrey. This strain was derived by crossing heterozygous nude mice from the department of Animal Genetics, Edinburgh with C₃H/he-mg SPF mice in the MRC colony. Hysterectomies were carried out into isolators and stock was fostered onto SPF C₃H/He-mg foster mothers. After testing for the presence of pathogens, offspring were introduced into the Centre's SPF Building. The building conforms to a category 4-star status and all parasites and important murine pathogens are absent.

Test matings were carried out by the MRC and the first homozygous SPF mice were born in mid-1972. As soon as sufficient homozygous (nu/nu) nude males were available all matings were made between heterozygous (nu/+) females and homozygous males. Homozygous matings were tried but with poor results (Festing and King, 1974).

Materials and Methods

The Breeding Nucleus

Upon receipt at the Cambridge laboratory 5 nu/nu males and 10 nu/+ females were placed in a sterile tissue culture booth and later transferred to permanent housing in a barrier conditions block. The animals were maintained under conditions designed to restrict contamination from

external environments, foodstuffs and personnel to a minimum. It was important to avoid contact with other animals, particularly mice, as previous work has shown that infections are rapidly transmitted, with fatal results (Sebesteny and Hill, 1974).

Housing and Maintenance

The floor area of the barrier room measured 9 x 12 feet. Cages were supported on a commercial mobile stand, (North Kent Plastics Limited) and a purpose-built 'handy-angle' type frame. Room temperature was held at 27°C but relative humidity was not controlled. Illumination was by natural daylight supplemented by artificial lights during maintenance periods.

The mice were caged in translucent polypropylene boxes with stainless-steel tops (NKP Limited). Two sizes were used, viz. 33 x 15 x 13 cm for small units and a 45 x 28 x 13 cm cage for breeding.

Autoclaved tap water was available ad libitum, dispensed from conventional glass or polypropylene bottles, fitted with ball-valve delivery tube. Originally, a 'conditioning mixture' containing tetracycline and multivitamins was given to the mice. After several days use however, such solutions were degraded by ultra violet light and allowed the growth of fungus in the bottles. Plain water was subsequently used.

Standard laboratory mouse breeding diet FFG(M), (E. Dixon and son, Ware) was provided ad libitum, previously treated with low pressure steam sterilisation. (More recently, the use of gamma-irradiated diet of the same type has been introduced.) A bedding material of wood shavings, sterilised by autoclaving at 30 psi for 10 minutes was used and was changed once a week. Sterile cotton-wool was provided for nest

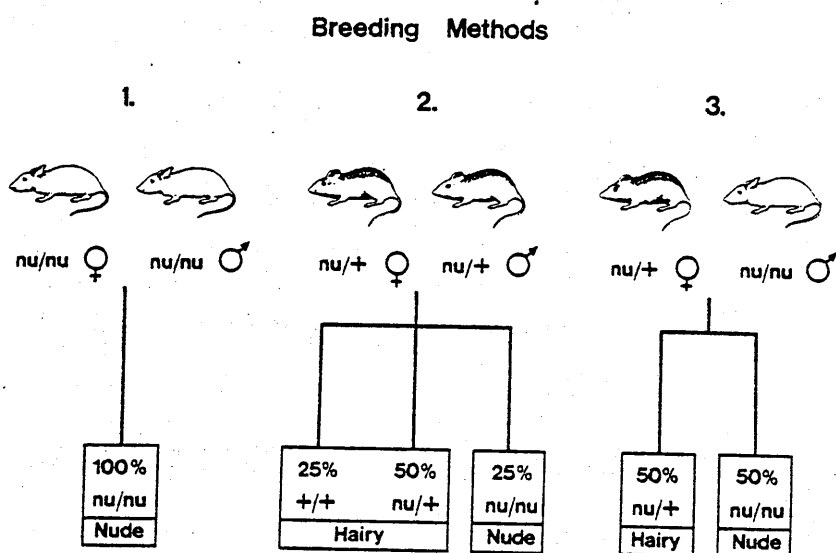


Fig. 2. Nude Mouse Breeding Methods

1. Homozygous matings are very unreliable due to low fertility and inadequate maternal nursing behaviour.
2. This method results in low production. Twenty-five per cent of normal offspring do not carry the nude gene and test matings must be carried out.
3. This method is reliable and results in a fairly high nude weaning rate and was used in this study.

building and cages were cleaned and sterilised by autoclaving at regular intervals.

Breeding

Standard Method 1.

Two or three phenotypically normal nu/+ female mice, 8-10 weeks of age were housed together and allowed to synchronise in pro-oestrus. After about 5 days they were placed in separate cages, alone or in pairs and mature nude nu/nu homozygous males were added. After a brief series of investigative and precopulatory actions, the male was usually accepted by the female and in a few cases exceptionally virile males attempted mating within 15 minutes of caging.

Harem Method 2

Four nu/+ females were caged together as described for method 1. A nude male was added to the cage and left to mate with each female. Towards the end of pregnancy, each female was placed by herself in a separate box. Fresh sterilised cotton-wool was added for the production of individual nests.

All cages were cleaned and fresh sawdust added immediately prior to parturition. This ensured that nests were not disturbed for the first 5 or 6 days part-partum.

Selection of Animals for study

Homozygous males of approximately 8-10 weeks of age showing good growth rate, normal appearance and in healthy condition were randomly paired with heterozygous females of similar characteristics. Any animals

that displayed obvious abnormal or unsociable behaviour were not included in the study. As litters appeared, the date of birth was recorded on the cage and the pups were assigned to either a behavioural or morphological study as appropriate. Animals used for behavioural scans were selected and transferred to the observation box prior to parturition.

Results

Breeding Techniques

Nesting

Parturition invariably occurred during the night and births were normally uncomplicated. In the case of the standard method, the males helped to rebuild the nest and to keep the young warm. The hairy pups invariably grew much faster than their nude littermates.

Productivity

In most cases, post-partum matings occurred in permanently caged mice and the young were delivered close to the time of weaning the previous litter. In the use of the harem method, certain disadvantages were found. Many nude males were not able to routinely impregnate more than two females and some males were attacked and killed before mating had occurred. Undoubtedly, the main disadvantage in a small colony, is the absence of post-partum pregnancies.

A study of 23 litters in the main breeding room showed that at weaning (28 days), nude young constituted 38%, i.e. 3.5 nude pups per litter. The expected yield of 50% nude offspring was not reached.

Mortality and Pathology

Few regular deaths of adult mice occurred in either the breeding or experimental colonies. Infections that did occur were low grade and localised. Some mice in particular, developed purulent swellings over the eyes and nose, probably due to the absence of protective hair. Another problem was prolapse of the rectum which affected both sexes on occasions, not being restricted to older mice.

	Festing & King (MRC)	Morgan (Cambridge)
No. Litters	82	23
No. nu/nu weaned	302	81
% nu/nu	43	38
Av. Litter Size	8.5	9.1

Table 5
Comparison of Nude Mice Productivity

The expected proportion of nu/nu young is 50%, so there is indication of increased pre-weaning mortality. Few deaths of nu/+ mice occurred.

Results for the Cambridge colony are therefore very similar to those obtained by the breeding source; i.e. MRC Laboratory Animal Centre, Carshalton, London.

Discussion

The productivity results for the original outbred strain at the MRC laboratories were described by Festing and King (1974). The expected proportion of nudes weaned from 82 litters was 43%, (3.2 nu/litter). Being less than the expected 50%, this suggests increased mortality of homozygotes prior to weaning, and involved animals aged less than 3 weeks.

Less than three percent of their homozygous males were infertile, but homozygous females, though fertile were unable to rear their young.

The output from their colony was roughly comparable with the output of an inbred strain with a poor breeding performance such as DBA/2.

Life span under SPF conditions exceeded 9 months; mortality between 3 and 10 weeks of age was virtually zero and there was no sign of the so called 'runting syndrome'. As the environment was SPF quality, infant death was presumably due to causes other than infection.

Breeding results for the Cambridge colony, derived from the same stock have been published, (Morgan 1976 & 1977) and show that similar productivity to the above could be obtained using simple barrier techniques. The study of 23 litters showed that at weaning (28 days) nude young constituted 38% (3.5 nu/nu) of the litter at weaning. This compares favourably with 3.6 nu/nu/litter for Festing and King (1974) who produced slightly fewer hairy pups per litter. Essentially then, the pre-weaning mortality rates were similar for both colonies.

CHAPTER 4

MEASUREMENT OF MATERNAL BEHAVIOUR

Introduction

This study considers a number of traits of maternal behaviour that may be demonstrated by the laboratory mouse from the period just prior to parturition, until the litter is weaned at 28 days post-partum.

Previous authors have reported that heterozygous nu/+ mouse mothers are able to conceive and rear mixed litters to weaning age efficiently. Few behavioural studies however, using either heterozygous or homozygous nude mothers, have been undertaken in order to characterise in detail such activities as nesting behaviour, nursing behaviour or mother-pup interactions, which are accepted measures of maternal behaviour (Noirot, 1969b).

Because nude pups invariably grow at a much slower rate than hairy littermates, it was important to carry out observations to confirm this finding and gain further information as to when and why a differential growth should occur.

Most of the work involved analysis of behavioural scan studies, using a simple check list for sampling behaviour, (see table 6).

Monogamous breeding pairs, consisting of a nude male and a nu/+ female were placed in a transparent plastic rodent cage with plentiful supply of sawdust/bedding a week prior to the expected time of parturition. A ball of sterilized cotton wool was placed in the centre

of the cage and the food hopper and water bottle were filled. The cage was placed next to a window that divided the barrier window from the central corridor as shown in Fig. 3.

Prior to each cage observation period, a diagram of the cage was drawn indicating the nest location which was evaluated using the criteria of Kapland and Hyland (1972). Observations were carried out for a 15 minute period in the morning, (08.30) and evening (17.30).

A check-list for scan sampling, (Simpson and Simpson, 1977) was devised which included a number of important items of behaviour; in the case of the female: time spent in nest, time nursing, feeding, etc. In an endeavour to quantify lactation behaviour, wherever possible, a note was made of individual pup feeding activities. Later on the time at which pup locomotor activity commenced, the change in mother-pup interaction etc, and the state of the nest were recorded on the check-list.

Item	Definition
On pups	Lies or sits in contact with the pups F M
Nest builds	Inside or outside of nest engaged in some aspect of nest building behaviour F M
In nest	Positioned inside the nest F M P
Nursing	Female was allowing the pups to suckle F
Suckling	Offspring obtaining milk from the mother: in nipple contact P
Licking pups	Female was licking or grooming her pups F (M)
Retrieves	Returns pup to nest F (M)
Eating	Nibbling at Food pellets F M P
Drinking	Drinking from the water container F M (P)
Resting	Lying motionless either inside or outside the nest usually with the eyes open but not involved in any other form of behaviour F M
Active	Moving about the cage at the moment of observation F M

F : item on check list for female parent

M : item on check list for male parent

P : item on check list for pups

The position of the young (inside or outside the nest) was also recorded together with any form of behaviour not catered for by the code.

Table 6. Behavioural Scan Checklist
items used in this study.

At each 1 minute interval during the 15 minute observation period, a scan summary was recorded using a prepared sheet according to the check list items above.

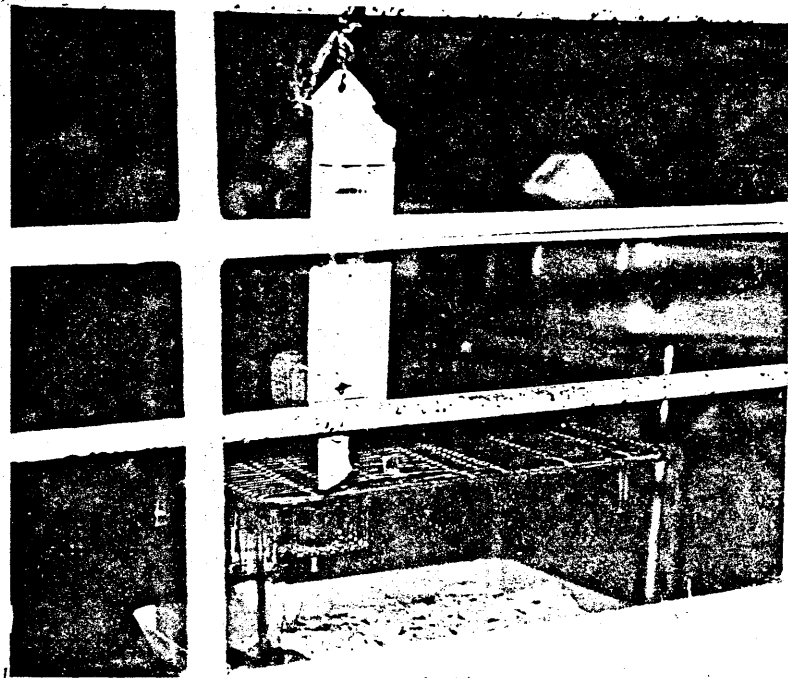


Fig. 3. Behavioural Scan Observation Technique

The test animals were maintained in a transparent perspex box on a shelf, near to a thick glass observation window, which separated the animal room from the corridor.

Fluorescent lamps illuminated the room and the corridor was kept dimly lit. A 15 minute observation could be carried out without disturbing the test animals.

Observation	No. Litters
Experiment 1 : Nest Building Behaviour	2* + 1
Experiment 2 : Nursing Behaviour	3* + 1
Experiment 3 : Female Activity	3*
Experiment 4 : Female Eating Behaviour	3* + 1
Experiment 5 : Mother-pup Interactions	3*

Table 7 Number of subjects used for the study of maternal behaviour

* indicates that the same litters were used for the category of behaviour described.

Experiment 1: Nest Building Behaviour

Materials and Methods

To determine the rate, quality and method of nest building a piece of cotton wool weighing approximately 4 g was teased into a small ball and placed in the centre of the observation box. The animals were then allowed to use this material for nest building without further disturbance. Two experimental boxes were observed.

It was noted that nests were often constructed under or near to the overhanging water and food containers, rather than other areas of the box. In order to test the hypothesis that the nest was purposely positioned under the hoppers, the following experiment was performed.

An nu/+ breeding pair was allowed to construct a nest as described above. Following parturition the box was examined and it was found that the nest had been constructed and maintained under the food container. The cage top was removed, turned around and then replaced so that the food and water containers were then in the opposite corner to the nest. During the following hour a record of behaviour demonstrated by the female was made.

Results

The nest size and form was generally preserved and nests could be rated 'good', with high sides and compact form, until, by about day 21 onwards they could become very flat, dispersed and offered less protection.

Daily examination of the boxes revealed that the animals tended to build and maintain their nests under or near to the overhanging food/water containers.

The results of the nest building experiment, where the cage top was reversed, suggests that this may not be a random result and may be associated with a particular nest building pattern.

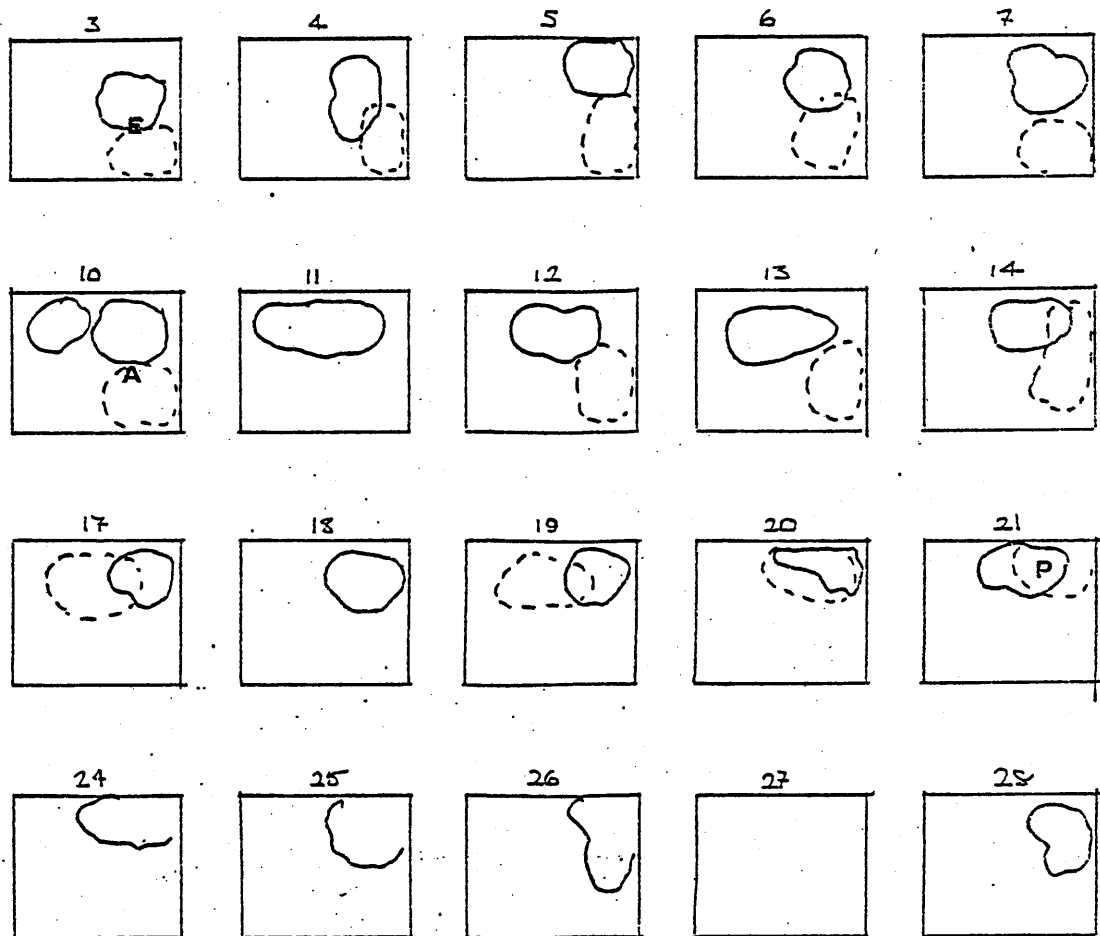
Stage 1: Immediately following reversal of the cage top the parent animals left the nest and began moving around the cage in an excited manner. Then the female re-sited the nest and litter. This procedure took approximately one hour to complete and subsequent important steps are shown in Fig 5.

Stage 2: The female burrowed under a high edge of the nest nearest to the newly positioned food/water hoppers. A large opening in the nest was made at this point and the pups were then pulled into this opening. At the end of this stage, the female left the nest, moved around the cage and took food and water.

Stage 3: The female approached the group of pups and (in a deliberate and previously unobserved manner), positioned herself over them.

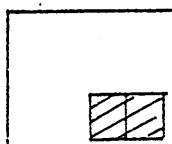
The crouching position was different to that used for nursing; the back was hunched, the body upright, and the tail was S-shaped, with the animal's rear in an elevated position. Each pup was then moved in the following manner: using her front legs the pup was pulled under the female's body and pushed through to the back legs. Next, using hind legs in a 'rugby player' movement the pup was propelled out from under the rear of the female, with some force, to a position some distance away at point 3. This continued until all pups had been safely moved to their new site.

Stage 4: Next, the female began to transfer the nest material, which was moved in the more usual manner, i.e. picking it up in the mouth and carrying it to the new site and depositing it over the pups. Finally a burrowing display was performed and sawdust was piled-up around the completed new nest. The entire sequence took 1 hour to perform.



Key:

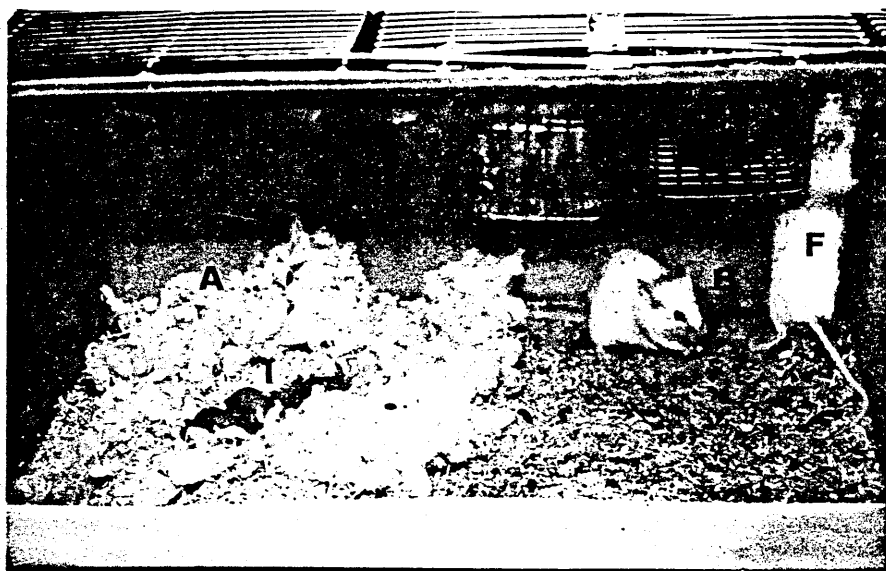
[Hatched Box] POSITION OF FOOD/WATER
 — NEST IN BOX 2
 --- NEST IN BOX 3



NEST CONDITION:
 E : EXCELLENT
 A : ADEQUATE
 P : POOR

Fig. 4 Nestbuilding: quality of nest post-partum

The position and quality of nests maintained by 2 heterozygous dams are shown at specific days post-partum. The nests were constructed, Maintained under or near (to) the water, and food containers and were rated 'excellent' for the first week and adequate until day 14 at which time they began to decline in quality and were 'poor' by day 21



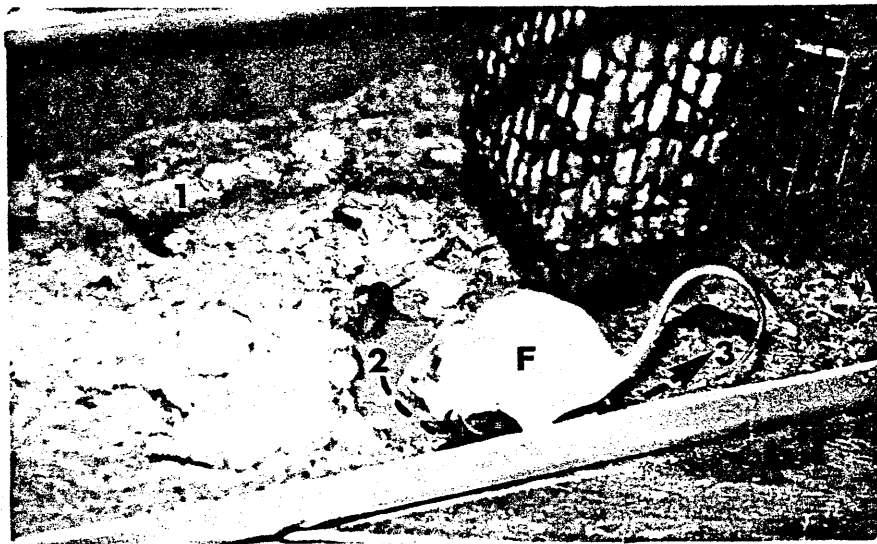
Stage 1: The cage top has been reversed so that the food/water containers no longer overhang the nest (1). This action immediately caused the animals to leave the nest and become active.



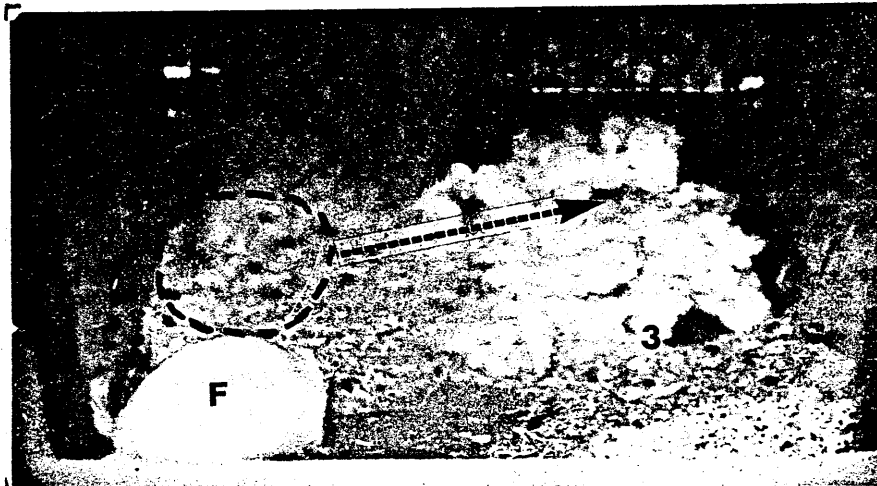
Stage 2: The female (F) burrowed under the edge of the nest and pulled the pups to an opening near to the newly positioned food hopper (2).

Fig. 5. Nest Building Experiment

The nest had been constructed naturally at point A, under the water/food containers. The cage top was then removed and reversed so that the containers were then in the opposite corner of the cage, (B). The resultant nestbuilding behaviour demonstrated by the female during a subsequent observation lasting 1 hour is described.



Stage 3: The female crouched over the pups and using an unusual 'scooping action', each pup in turn was pulled under her body and passed out to a new site at 3. This display continued until all pups had been transferred.



Stage 4: The female next transferred the nest material to cover the pups at the new site.



The completed new nest - under the food/water container.

Discussion

The results from the nestbuilding experiment suggest that phenotypically normal nu/+ mice display a high degree of normal nestbuilding behaviour.

Good quality nests were routinely produced prior to parturition and were well maintained until about 2 - 3 weeks post-partum, suggesting that maternal hormone levels during pregnancy and pup stimulation effects post-partum were of a high order.

The finding that the mother may selectively build a nest and also move an established nest to a protected site, i.e. the overhanging water/food containers, further suggests that the mice in this study display effective nursing behaviour. Priestnall (1972) confirmed that when food and water were supplied over the nest in this way, females caring for both large and small litters spent significantly more time in the nest than females caring for similar litters, when food and water supplies were outside in the cage.

The method of pup transfer observed in experiment 1 was very uncommon and had not been noticed previously under standard breeding circumstances. It is tempting to suggest that in covering the pups with her body and scooping them from under her to the new site, the female offered more (thermal) protection than if normal mouth retrieval actions had been used, when the pups would be left hanging in the air for longer periods.

Elwood (1975) reviewed maternal and paternal behaviour in the mongolian gerbil and showed that the male displayed a high degree of 'maternal behaviour'.

Similarly in this study it was found that a nude (and nu/+) male parent could spend considerable periods in the nest, gathered the pups under his body and assisted with nest building and retrieval activities.

Experiment 2: Nursing Behaviour

Materials and Methods

During the daily observation periods the time spent nursing was recorded. Whenever possible the mode of interaction between mother-pup and pup-pup was noted.

Results

During daily observations on three litters note was made of any nursing behaviour that occurred. Often however, nest material, and/or the positions of the male or female made continuous observations difficult. In one box sequential observations resulted in a detailed record from day 10.

Day 10: The nude pups ($n=4$) were positioned on the periphery of the suckling circle, experiencing great difficulty in obtaining milk; 5 nu/+ animals were successfully feeding.

Day 14: By this time one nude pup had died and one other was very thin and weak.

Day 17: Three out of the four nude pups had died; the remaining nude pup competed actively for a suckling position. The female moved from the pups and took up a new position in the nest. The hairy pups followed her and adopted a new position under her. The nude pup followed but after trying to locate the nipple unsuccessfully, finally sat by the male. During this 15 minute observation, the hairy pups were in contact for approximately 10 minutes, whereas the nude pup was never observed sucking.

Day 20: Nu/+ pups were in nipple contact - others were beginning to explore away from the nest. The nude pup was trying to locate the nipple. The female moved position; was quickly followed by nu/+ pups who resumed a suckling position. The dislodged nude desperately climbed onto the female's back and moved to her far side, but was unable to make nipple contact.

Day 22: Nu/+ pups in nipple contact. The female moved dislodging pups. Two nu/+ pups immediately regained nipple contact. The nude pup then searched for the nipple and gained hold, but soon lost contact. It desperately tried to regain contact but was pushed aside by a nearby hairy pup.

Day 24: Nude pup dead.

Discussion

Throughout the observation periods, the female spent much of her time in the nest area. During the early stages i.e. 1-7 days post-partum, she would take-up a continuous crouching position over the pups.

The female spent the least time in the nest during day 10-13, which coincides with the period when the difference between hairy and nude pups, in terms of body weight increase starts to become apparent.

The scan observation results reported above suggest that at all ages, the hairy pups were seen to be in nipple contact for long periods, whereas the nudes were often unable to locate the nipple. Certainly, if the mother moved to another position within the nest, nude pups alone, were invariably unable to continue feeding; all nudes in this experiment died before weaning.

It seems that simply the presence of the nursing mother is not sufficient to ensure that nude pups obtain adequate milk in a normal nesting situation. More importantly, failure of pups to thrive may be the result of their inadequate nipple search and suckling stimuli, early post-partum.

Experiment 3: Female Activity

Materials and Methods

Periods when the female was away from the nest (during 15 minute observation times) were recorded using the scan sheet. Such activity could include locomotion, climbing and eating and drinking actions.

Results

Usually female activity included: movement around the cage, burrowing in sawdust, climbing the food hopper and eating/drinking.

The percentage of observation time that the female ate or drank was scored and tended to be low in the early and late stages post-partum. The highest frequency for feeding behaviour occurred on the day that the female was most active, i.e. day 12. If the female had been fertilised at post-partum oestrus, then this would usually result in a return to nest building activities by day 28.

Discussion

The effects of litter size on the time spent by the mother in nursing and licking pups and in eating and drinking have been discussed by Priestnall (1972). He suggested that discomfort produced by excessive contact and interference from the litter or from the extra stimulation of the mammary region during suckling could also result in females rearing larger litters ($n=8$) leaving the nest more often.

In this nude mouse study, as the mother spent a larger percentage of observation time on day 12 and 13 in out-of-nest activity, it could be suggested that the pups were feeding efficiently and inducing fatigue in the mother, which was reflected in a reduction of nesting

behaviour at that time.

This period coincides with the beginning of a fall in the rate of body weight increase for nude pups found at that time. It is possible that although hairy pups were maintaining a good level of nutrition, nudes were experiencing suckling problems which were exacerbated by the absence of the mother from the nest.

Experiment 4: Feeding Behaviour

Materials and Methods

Using the scan sheet, the time spent feeding during a 15 minute observation was recorded. In addition, in the case of one female, the daily weight of food consumed was recorded.

Results

The period (%) of time that the female was observed feeding or drinking was plotted against days post-partum.

Peaks in female activity away from the nest were found on day 3, day 10, 12, 13 and day 20 and day 24.

On day 12 the female spent the least observation period in the nest; nearly all of this time was spent in eating and drinking activity.

A quantitative experiment was performed to assess the actual weight of food consumed following parturition. The daily weights are plotted in Fig. 7. This shows that the quantity of food consumed by a single nursing female increases quite steadily after the birth of the young, as reported by Smart and Silence (1977) for albino mice. By 2 weeks post-partum the female's daily intake of food had increased to $2\frac{1}{2}$ times that of the pre-birth daily consumption.

At 3 weeks post-partum the graph suddenly peaks, probably indicating that the offspring were beginning to take solid food. This corresponds with the behavioural scan studies which indicate that at about this time the hairy pups become very active, move around the cage, climb the food hopper, etc.

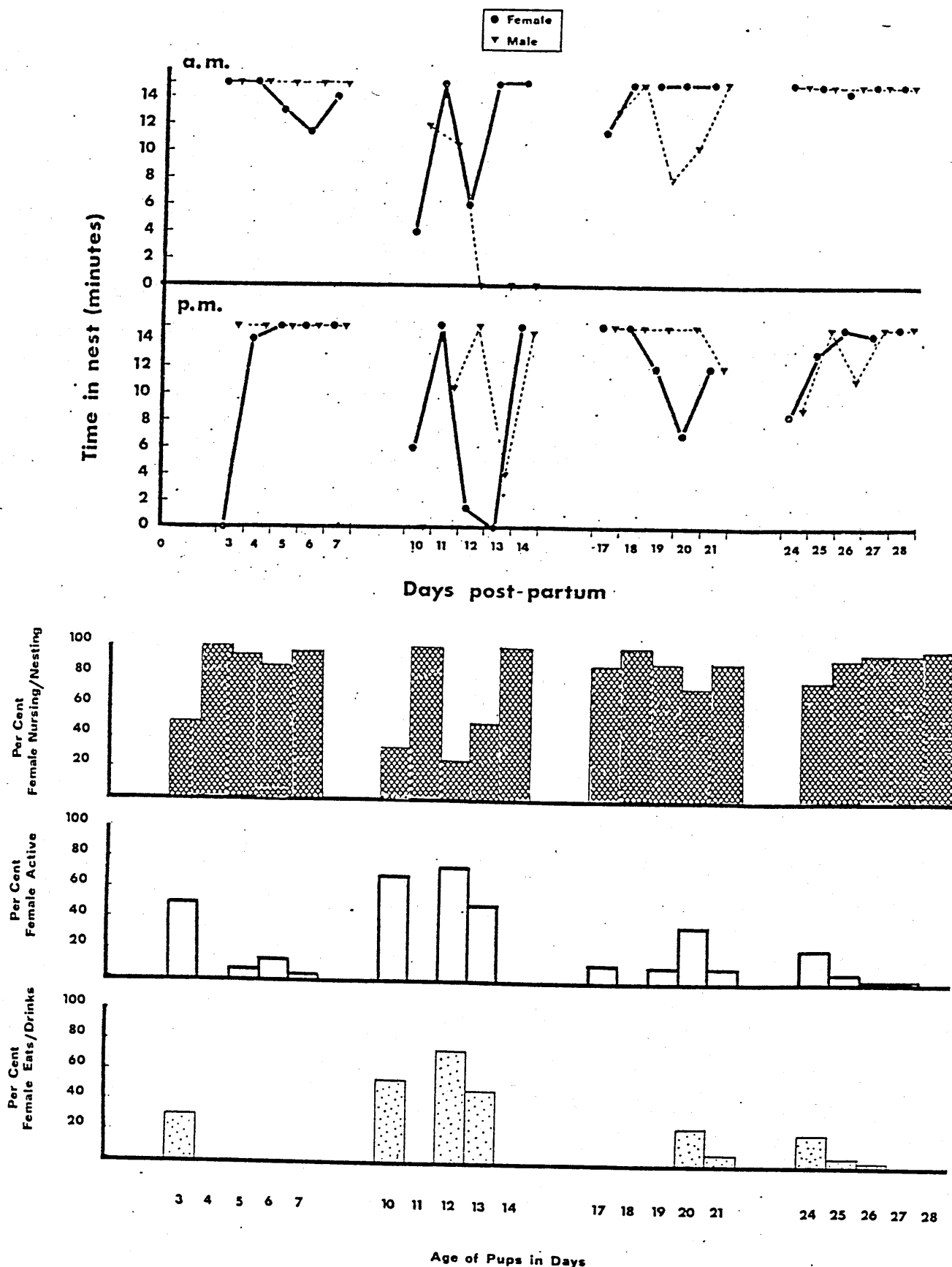


Fig. 6. Behavioural Scan Results

Observations were carried out on 3 litters, for 15 minutes twice daily, Monday to Friday at 8.30 a.m. and 5.30 p.m.

The top figure shows that both the female and male spent much of the observation time in the nest during weeks 1, 3 and 4 post-partum. The lower figure reveals that the high out-of-nest behaviour during the second week, particularly involving the female, was related to a great increase in cage activity associated with eating and drinking, (expressed as a % of total observation time).

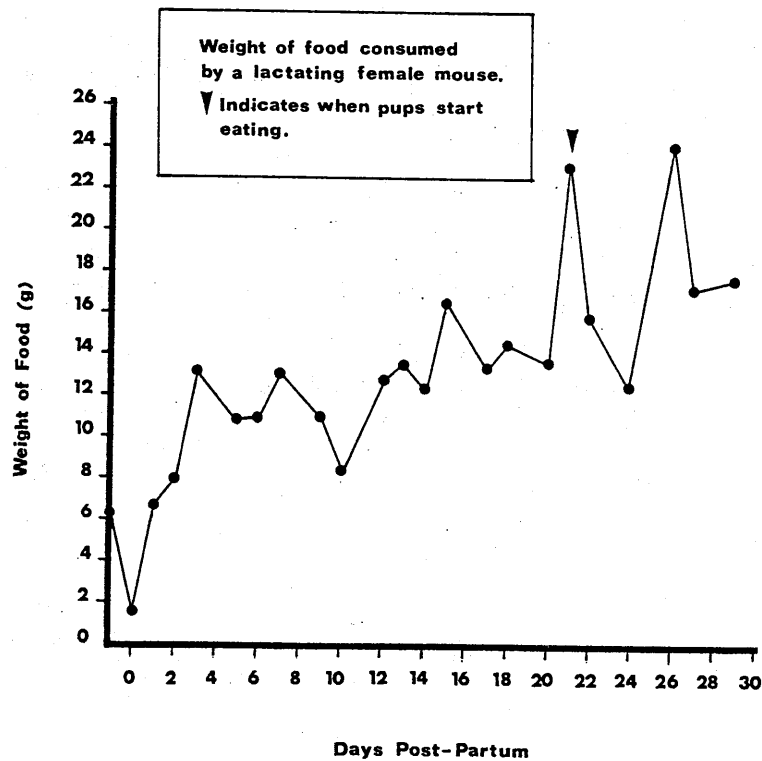


Fig. 7. Female Eating Behaviour

On the day prior to parturition the weight of food eaten by the pregnant mouse was found to be 6 g.

On the day of birth the food consumption decreased significantly to just over 1 g.

Eating activity then steadily increased over the next 18 days, with a mean daily intake of approximately 12 g.

By day 26 the daily food intake had greatly increased indicating that pups had begun to eat solid food in large quantities.

Discussion

Priestnall (1972) showed that eating behaviour was the most frequently observed non-maternal behaviour of mice, when out of the nest. Eating appeared to reach a peak incidence at about day 14.

In this nude mouse study a similar result was obtained with females eating and drinking on days when they appeared to be most active, i.e. day 12 and 13.

This quantitative study for a single lactating female (Experiment 4) shows that the quantity of food eaten increases steadily during the 3 week period following the sudden fall in food consumption on day 0 i.e. day of birth of litter.

The studies by Smart and Silence (1977) showed that under-nourishment of maternal mice resulted in considerable nestling mortality, with fewer young being neared to weaning.

The results of this experiment suggest that heterozygous nu/+ females obtained adequate nutrition during the suckling period.

Experiment 5: Mother-pup Interactions

Materials and Methods

Using descriptive means, a sequential study of three litters was made of the developing physical relationship between mother and offspring, until their separation at weaning.

Results

The following stages (in this development) were observed:

Day 1 - 5: In this period nursing and suckling predominates. The animals soon began to locate the female and compete for nipple positions. Sleeping was 'fitful' and the litter reorganised itself at regular intervals. Nu/+ (hairy) animals were more efficient at locating the nipple than nude pups and could be easily identified because of their greater size, from day 4 or 5 onwards. The development of the righting reflex and similar actions probably occurred during this period.

Day 6 - 12: Sleeping and suckling still predominate, the animals may 'twitch' and shuffle into more comfortable positions. During this period the female may leave the nest at frequent intervals and may do so very suddenly, pulling pups out of the nest attached to the nipple. The pups begin locomotion at this time and may find their own way back into the nest or may be retrieved by the female. If the pups were deliberately removed from the nest, retrieval actions were rapidly initiated. Although the female was mainly responsible on two occasions the nude male parent engaged in such actions. It appeared that nude animals were retrieved more often and at a greater age than the hairy animals. The female also engaged in deliberate and progressive licking actions of the pups' face, body and genital regions.

The nu/+ animals were easily differentiated from the nude animals on account of their physical development, weight gain and size. Powers of locomotion increased steadily during this period and in open field tests quantitative data was obtained. Not only did hairy pups move further than nudes but in some cases they changed from crawling in circles to straight-line walking before nude littermates. By day 12 nu/+ animals had developed a complete hair covering and long whiskers, whereas nude animals were hairless and whiskers were essentially absent or sparse, short and crinkled.

Day 13 - 20: Eye-opening in hairy animals commenced at about D13 whereas this may be delayed for two further days in the case of nude pups. The nu/+ animals moved freely around the nest and on D17 were observed making their first exploratory movements away from the nest, which were to and fro darting movements from nest to corners of the cage.

Day 21 - 28: At about day 21 - 24 the pups entered a period of increased activity. This was most noticeable in litters where good nutrition had favoured rapid growth and development. Sniffing, running, jumping and climbing all occurred in a jerky jumbled sequence. Some pups were observed drinking.

In one litter studied where nude animals had developed well, they too followed similar activity patterns. In other litters examined (3) the surviving nude animals remained in the nest.

Day 24: Nu/+ pups were observed licking and grooming themselves. Both parent animals were engaged in similar activity.

Day 25: A nu/+ animal was observed tumbling over in the nest and fell onto the male nude parent who nipped the pup in response. The pup moved to another part of the nest. Pups frequently approached the food

hopper to search for food fragments or climbed the hopper to eat. Nude pups, in litters where good nutrition had favoured development, were also mobile at this age, but continued to spend much time near the nest. In some cases, however, they would demonstrate a 'game' of mounting the nude male's back, they would be transported around the cage and would then hop off, back into the nest. This 'game', when it occurred, concerned the nude animals and the male parent only.

Day 27: Female may begin nest building.

Day 28: Pups spend most of the observation time climbing, scratching, preening, etc. Pups were weaned.

Discussion

The development of behaviour in the heterozygous nude mouse post-partum, follows the general pattern seen in the rat (Bolles and Wood, 1964), the gerbil (Kapland and Hyland, 1972) and the mouse (Scudder et al., 1967).

During the perinatal period the mother spends much of her time in the nest with the litter in a nursing position. The first few days however, (certainly day 3), proved to be a period of adjustment between the female and her litter and less time was spent nursing on day 3, than on any other day except days 10 and 12.

If the mother left the nest the huddled pups became unsettled, possibly because of disappearance of maternal odour from the nest (Kapland and Hyland, 1972). Although pups were generally quiet or asleep while nursing and huddling, there were periods of activity as they attempted to obtain a nipple.

The mother tended to be responsive to the pups and would readily retrieve any pups that left the nest, possibly showing a greater interest in this respect, for nude pups, which may have been moderated by ultrasonic calls (Noirot, 1966).

During the period Day 6 - 12, the hairy pups had matured rapidly and were able to move from the nest and by the end of the second week of lactation began to take a more active role in the initiation of nursing, probably reflecting their increasing co-ordination and strength and ability to follow the mother if she changed position.

Scudder et al. (1967) studied six different strains of mouse and found a significant variability in maternal interest, which was generally correlated with development and ceased when the young opened their eyes and achieved some degree of co-ordination and independence.

Similarly, during the third week in this present study, interest in the hairy pups began to wane as they spent increasingly more time in locomotion; nude pups were left behind in the nest where they may have been subjected to some maternal interest, e.g. licking, which correlates with their immature form and lack of independence. By day 28 all pups had developed independent behaviour and maternal (and paternal) interest was minimal.

CHAPTER 5

Measurement of Pup Development

Introduction

The present study deals with the postnatal development and behaviour of athymic nude mice and hairy controls during the period from birth until 28 days of age.

Since Macdowell (1930) many workers have investigated the effects of altered litter size on the growth and development of rodents; increasing litter size permanently reduces body growth and reduction of litter size enhances growth and development (Teicher and Kenny, 1978).

In this present study cross-sectional and longitudinal growth data was obtained and a number of experiments are reported where hairy pups were removed from the litter at various times post-partum. In some experiments such pups were then returned to the home nest, following a period of 3 days separation.

Previous work has shown that nude animals do not attain the size of nu/+ mice, whether they are reared in conventional or Specific Pathogen Free environments (Poiley et al., 1974). Recent work also suggests that the growth of athymic nude rats is reduced to about 60-80% of their heterozygous littermates (Festing et al., 1978).

In order to compare this present study with others it was necessary to determine the body weight curves for the strain of mouse

under investigation. Both cross-sectional and longitudinal results were obtained.

Subsequently, experiments were undertaken in which numbers of hairy mice were removed from the nest at various times post-partum. In other experiments such animals were kept isolated for 3 days and were then returned to the home nest and weighed at regular intervals.

The effects upon the body weight curves for both groups of animals are reported.

Retrieval of mouse pups to the nest by the mother is important in the early stage post-partum. Such retrieval and associated nursing actions are a response to ultrasonic calls originating from the pups.

A preliminary experiment on ultrasonic calling in nude mice is reported which compares the call rate and duration of calls elicited by nudes and hairy controls, when such pups are separated from the mother in low temperature surroundings.

Because the rat has been used as an animal model in many experiments concerned with developmental retardation it has been necessary to describe the normal postnatal development of locomotion in the laboratory rat in considerable detail (Altman and Sudarshan, 1975).

Similarly, because the mature hairless mouse has been shown to display reduced locomotion in an open field test, this study has considered the locomotion development in the 'standard' nude mouse and has compared exploratory behaviour of randomly selected mature nude animals (from a standard breeding colony) with hairy controls.

Normal Growth curve (weight):	No. of Litters
Longitudinal study	3
Cross-sectional study	>6 (50 animals in each group)
Suckling behaviour	2
Litter manipulation Expts	7
Ultrasonic vocalisation	1
Development of locomotion:	
Longitudinal study	1
cross-sectional study	5
Exploratory behaviour in weaned animals	10

Table 8 Number of subjects used for Pup
Behavioural/morphological studies

Experiment 6: Standard Body Weight Curves

Materials and Methods

For the cross-sectional study two groups of mice, consisting of 50 nudes and 50 hairy littermates, were randomly selected from a standard breeding room.

The animals in the age range 2 - 28 days of age were weighed once only on a laboratory animal balance to 0.01g.

Pups from 3 further litters were weighed on a daily basis in order to establish a longitudinal growth curve for animals maintained within the same maternal nest.

Results

From birth until day 7 the curves for both groups run parallel but after day 8 the growth curve for hairy pups begins to steepen. The nude pup growth curve however, dips slightly at day 8 and thereafter climbs slowly.

From day 12 - 18 the nude pups gained very little body weight, compared with the hairy sibs, who increased in weight by more than 2g during this period.

Following this break, the nude pups continued to increase slowly, reaching a mean body weight of 8.5g at 28 days of age. The average weight for hairy pups from the same litters was 17g at weaning.

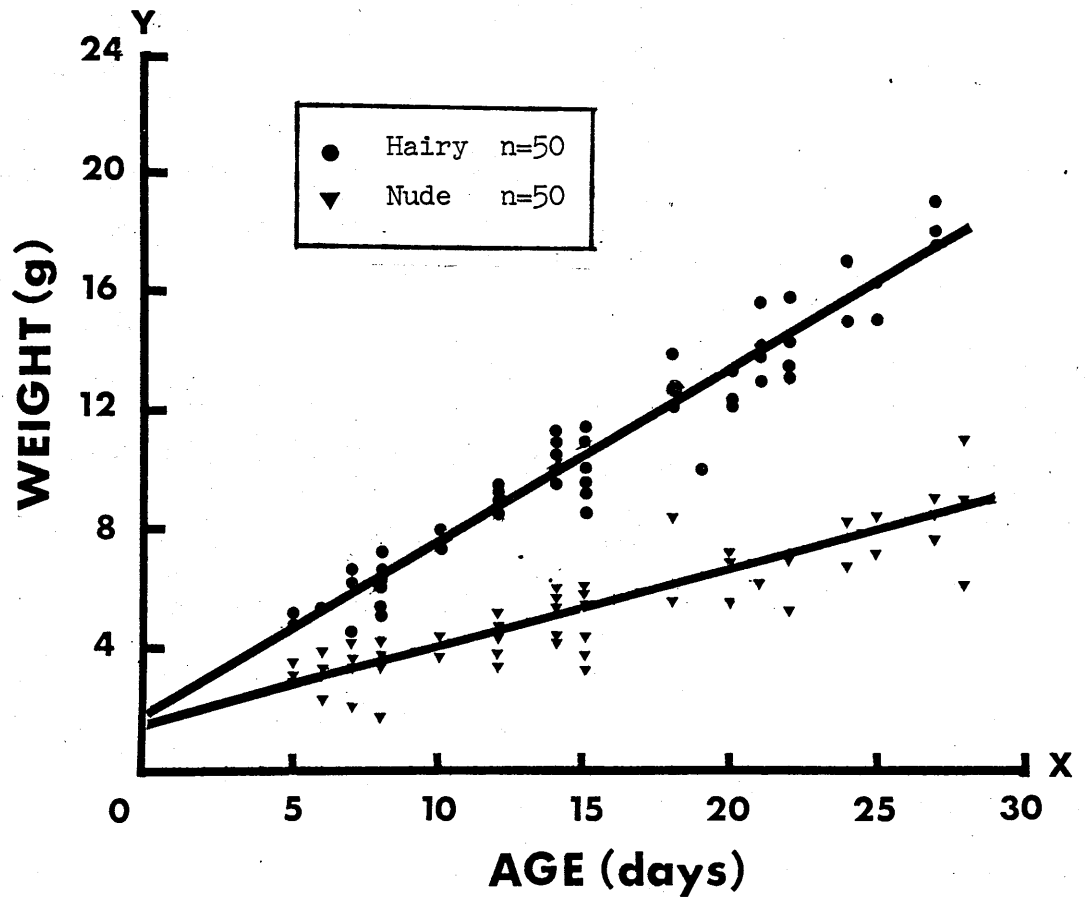


Fig. 8 Normal Growth Curve: Cross-sectional study.

Two groups of mice each consisting of 50 nude and 50 hairy littermates were taken at random from a main breeding room and were weighed once only on an animal balance, to 0.01g

Regression analysis was carried out according to the equation:-

$$y = mx + b$$

$$\text{where } m = \frac{N \sum x_i y_i - \sum x_i y_i}{N \sum x_i^2 - (\sum x_i)^2} \quad \text{and } b = \frac{\sum y_i \sum x_i^2 - \sum x_i \sum x_i y_i}{N \sum x_i^2 - (\sum x_i)^2}$$

	<u>Hairy</u>	<u>Nude</u>
Intercept values:	1.66	1.52
slope :	0.58	0.25
<u>value for x</u>	<u>values for y</u>	
5	4.58	2.78
10	7.51	4.05
15	10.43	5.32
20	13.35	6.57
25	16.27	7.85

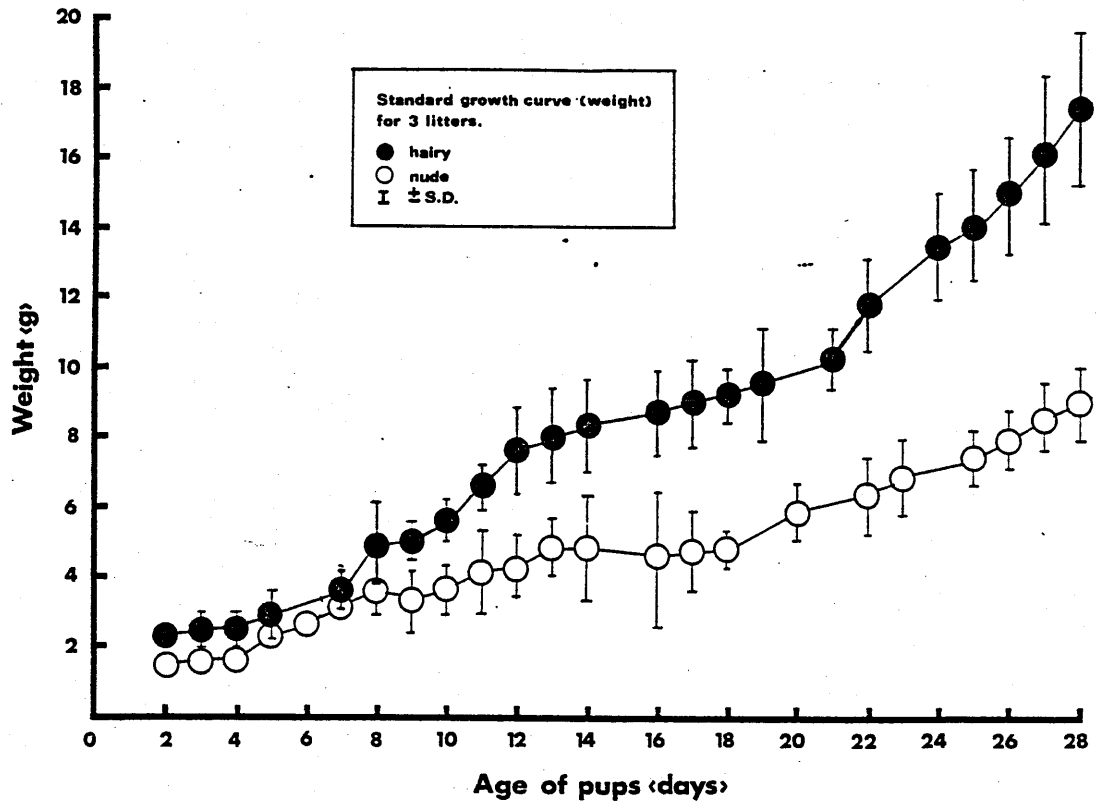


Fig. 9. Normal Growth Curve: Longitudinal Study.

Hairy and Nude pups from 3 litters were weighed at regular intervals on an animal balance to 0.01g.

The growth curve for hairy pups begins to steepen at day 8. The curve for nude pups runs parallel until day 12, when it flattens-out for a further 6 days, until day 19, when growth is again accelerated. At weaning, nude weights were approximately 50% of the body weight achieved by hairy littermates.

The probability levels of difference between groups was determined using Student's t Test:

Day 2 - 7	=	$P < 0.01$
8	=	$P < 0.05$
9 - 10	=	$P < 0.01$
11 - 28	=	$P < 0.001$

Discussion

Poiley et al. (1974) reported that in Nu/Nu - Balb/c/A Bom Cr mice weaning weights averaged 12.2g for nudes and 17.9g for hairy pups.

Rygaard (1973) showed that by 6-8 weeks of age the difference in body weight between nude and hairy mice was becoming less marked, until at 2 months of age weights were comparable (Fogh and Giovanella, 1978).

Scudder et al. (1967) found a break in the growth curve for Mus Musculus after the first week and also at 14 days of age.

Both nude and hairy pups in the present study showed a slight flattening of their growth curve after the first week, probably due to a reducing milk supply. At 14 days however, the nudes displayed a noticeable fall in weight gain which may be due to less milk being available and their inability to progress to an adult diet.

The results from a study of 7 litters by Rygaard (1973) showed that nudes in standard mixed litters suffered a marked retardation of growth, which started on the 13th day post-partum. Growth did not accelerate again until about day 21, which compares with day 12 and day 19 respectively in this present study.

Experiment 7: Suckling Behaviour

Materials and Methods

During daily observation periods described in Chapter 4, note was made of any pup suckling behaviour that occurred.

Particular note was made of the spatial relationship of the pups to the female, any weakness in suckling and the ability of pups to regain nipple hold following movement of the mother.

A series of daily summaries of mouse behavioural scan observations are presented, using two separate litters.

Results

In box 2, from day 10 - 18 the hairy pups were in nipple contact for a large percentage of observation time. Nude pups were not seen suckling during this period. From day 18 - 28 the hairy pups progressed to solid food. The 2 nude pups in this litter had died by day 21.

Similar observations on another cage (box 3) showed that nudes tended to lie away from the other pups on the periphery of the 'suckling circle' and were incapable of rapidly locating the nipple when the mother changed position. Hairy pups were always able to regain nipple hold. Nude pups were generally dominated by hairy sibs when attempting to make nipple contact. The nu/+ animals were very robust and able to push the smaller nude pups aside.

However, it appeared that the nude pups were experiencing difficulty in locating the nipple area and often wasted considerable time and energy in investigating non-mammary areas. On a few occasions nude pups tried

to suck from the ventral surface of the male (nu/nu) parent. If the female changed nursing position hairy animals were quick to follow and regain nipple contact. Nude pups were often unable to copy this.

Box 2

Mice Behavioural Scan - Suckling Activity (15 min. observations)

Day	Activity		Comments or Diagrams etc.
	Nu/+	Nude	
10	suckling	on periphery of suckling circle	
11	"	"	
12	tend to move frequently in nest	seldom move	
13	actively move within nest area	"	disorganised nest
14	well positioned on nipple	2 pups in far corner of cage	
17	1st pup to leave nest; goes to food - returns to nest immediately. Other pups look out from nest	1 remains in nest 1 dead?	Parents active
18	1 pup sucks actively; 1 pup follows female as she leaves nest	pup unsuccessfully seeks nipple	
19	several animals wander on periphery of nest	remains in nest	
20	pups actively move in nest	little movement	
21	" (1st adult-like actions)	pup dead	
24	within nest area: sniff at parents; scratch around floor or nest		
25	Burrow under cotton wool; push nest outwards; moves short distance outside nest; sniffing at parents; jumps up wall; smells floor debris; climbs food hopper, bites at food.		
26	very active: pups move in/out of nest; individual preening; 1 pup drinks;		

Day	Activity		Comments or Diagrams etc.
	Nu/+	Nude	
26	eats food debris under hopper; roof climbing; hangs from food hopper and bites bars.		
27	considerable time spent self-preening; one pup pushes sawdust out of the the nest		
28	climbs food hopper; eats; scratches floor; self- preening; pulls cotton wool to nest; scratches; drinks; preens litter- mate		

Box 3

Mice Behavioural Scan - Suckling Activity (15 min. observations)

Day	Activity		Diagrams etc.
	Nu/+	Nude	
6	Able to follow mother in nest	Seldom move	
7	pups actively compete for nipple	lie still	
10	in sucking position	on periphery of feeding circle	
12	huddle together	2 nudes lie away from littermates	
14	sucking; healthy condition	1 nude lies dead; other thin & weak does not move.	
17	in sucking position. ♀ moves followed by individual pups who take up new sucking position.	1 nude fights for nipple position; pup follows other nu/+ pups and tries to suck. Unable to locate nipple, finally lies by ♂	other nudes dead
19	pups in contact with nipple	the nude pup tries to push under ♂ as if feeding.	
20	pups move freely around cage; often returning to nest area. Some pups in nipple contact ♀ moves position, followed by nu/+ pups.	Nude endeavours to suck; dislodged by ♀, desperately climbs onto ♀ and moves to her far side but unable to make nipple contact	
21	constant activity; climb food hopper; leave/return to nest	nude remains in nest	
22	pups in nipple contact. ♀ moves dislodging pups. 2 nu/+ regain hold	nude searches for nipple, gains hold of nipple; loses hold; makes desperate attempt to gain hold - forced aside	
24	nu/+ starts to find nipple succeeds and pushes nude aside.	last nude dead	

Discussion

The results confirm the observations of others (Arts, 1970; Heatherington and Hegan, 1975) that nude pups have some difficulty in competing with active hairy pups for maternal milk.

If the mother changed position, the nudes were instantly dislodged from the nipple and were not easily able to regain a suckling position.

It is apparent that nudes have difficulty in locating the nipple region; on one occasion a nude pup, having failed to obtain milk from the female, then moved to the male parent in a desperate search for a mammary gland.

The work of Teicher and Blass (1978) on 4 - 5 day old rats showed that pups were unable to locate the mammary gland after the glands had been washed to remove an 'olfactory cue'.

Similar work by Hofer et al. (1976) also showed that the presence of an olfactory cue (possibly lipid substance) on the mothers mammary glands was essential for the orientation and attachment by two week old rat pups. Infants which had been rendered insensitive to olfactory cues were unable to attach to the nipples of an anaesthetised lactating female and when housed with their own unanaesthetised mother, they lost weight and often died.

Teicher and Blass (1978) reported that rat pup saliva is a sufficient, although not necessarily the exclusive source of olfactory cue. Analysis of both the nipple-wash extract from the lactating mothers and pup saliva residue by thin layer chromatography, indicated that they contain similar components.

These preliminary observations on suckling nude pups suggest that they may display depressed olfactory senses and/or may be unable to deposit a vital chemical cue on their mothers nipples, possibly due to defective salivary glands. (Holmes and Mason, 1974).

Experiment 8: Removal of all nude or all
hairy pups from the litter

Materials and Methods

Two litters were assigned to this experiment at day 0. In the first litter, all nude pups were removed from the nest at day 4 and sacrificed. The remaining hairy pups (n=5) were weighed on a regular basis until weaning at day 28.

In the second litter, all hairy pups were removed and sacrificed at day 5 and the remaining nudes (n=6) were then weighed at regular intervals.

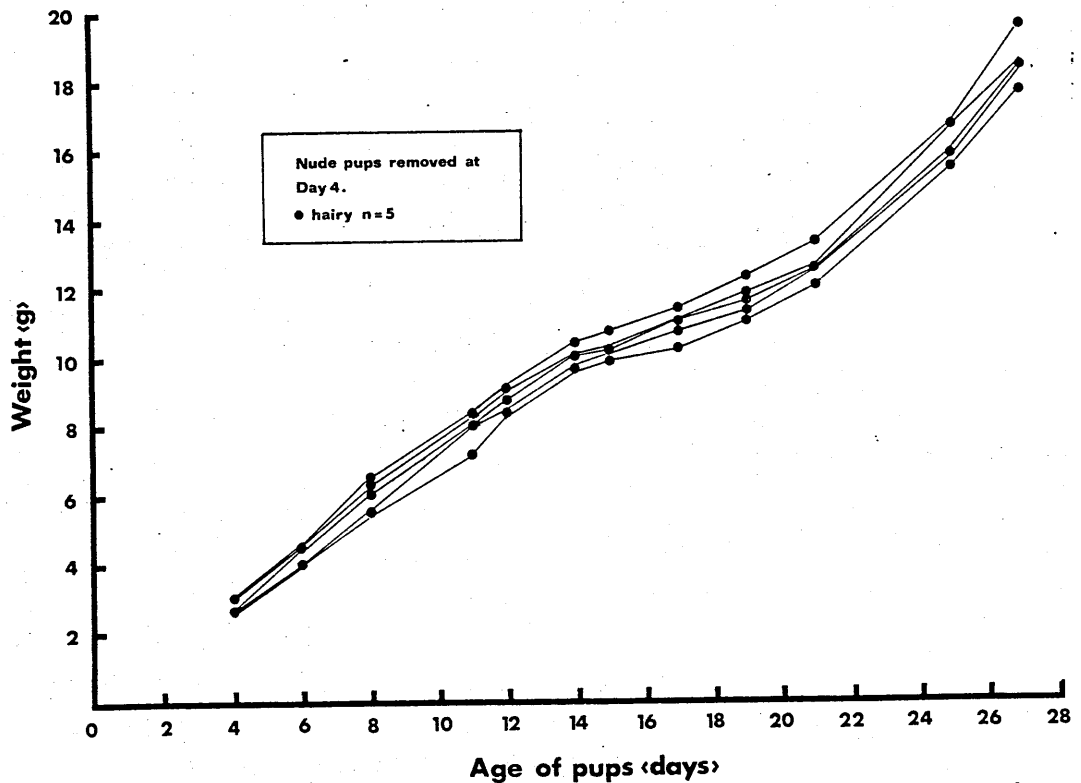
Results

In the absence of nude pups, all the hairy sibs in the first litter were reared to weaning successfully. Body weights for all 5 pups at weaning were very similar and the growth curve closely followed the average curve for mice in standard litters. The hairy pups did not benefit nutritiously, from the absence of nude pups.

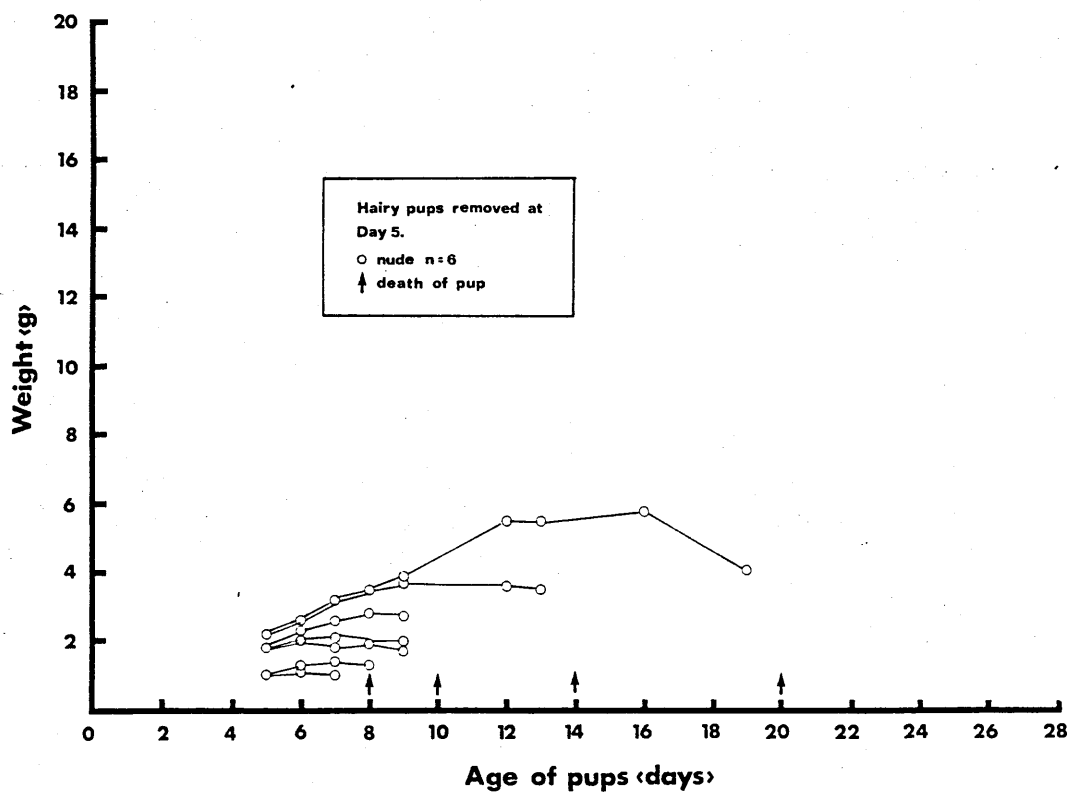
On the day of commencing the experiment with litter 2, i.e. day 5, the 6 nude pups were fairly similar in body weight. By day 7 four of the pups experienced individual degrees of rapid weight loss, resulting in deaths of nudes on day 8, 10 and 14 post-partum.

The remaining nude pup was able to maintain a growth curve which was consistent with good nutrition until day 12. At this time, the curve began to level out and over the next 4 days no weight increase occurred. From day 16 the body weight of this pup fell steeply and the pup was found dead on day 20.

The results are consistent with almost total deprivation of milk-intake by the nude pups which correlates with removal of hairy pups from the nest at day 5. From day 0 - 5, in the presence of hairy pups, the nudes had increased in bodyweight in line with the standard curve, (see Fig. 9.). It is also evident that the problem is an individual one, suggesting that individual pups experience greater or lesser problems than other nude littermates.



Litter 1: Removal of all nude pups shortly after birth, (day 4). The individual pups gain weight at a similar rate and reach, but do not increase above, the standard body weight for age, at weaning.



Litter 2: Removal of all hairy pups shortly after birth, (day 5). In the absence of hairy pups the nudes commence to lose weight and 4 pups die during the next 5 days. Individual pups suffer different degrees of suckling problem; all pups died before weaning.

Fig. 10. Removal of all hairy or all nude pups shortly after birth

Discussion

This simple experiment appears to support the finding of others (Giovannella and Stehlin, 1973; Artz 1972) that nude mice may require the presence of hairy pups for successful development, although this has not been the case for all studies, (Rygaard, 1973) .

It is clear however, that the majority of nude pups had achieved standard body weight by day 5 but all immediately suffered nutritional problems upon removal of hairy pups from the litter. The remaining nude pup, although adequately nourished up to day 12, then experienced similar problems which also coincided with the period of low nursing scores, found for some lactating mothers, i.e. day 11 - 13. It can be suggested that lack of pup stimulation effects on mother, coupled with high away-from-nest activity level for the mother, together resulted in decline in weight and finally death of the remaining nude.

The presence of hairy pups may be essential for producing stimulatory and milk-ejection oxytocin effects upon the mother. Too many robust hairy mice however, may have adverse effects upon the nude sibs, who appear to experience difficulty in locating and maintaining hold on the nipple. Overactivity on the part of too many hairy pups will tend to tire the mother and may cause her to leave the nest more often and so exacerbate suckling difficulties for nude pups.

A number of litter reduction experiments (Macdowell et al., 1930) have shown the benefits of reducing the number of suckling pups in the litter; application of this technique in the case of hairy pups during the first 3 weeks of life may have beneficial results, for the nude sibs.

Experiment 9: Removal of all hairy pups at Day 6

Materials and Methods

A litter of pups was assigned to this experiment at day 0. Regular weighings of all animals were made, until day 6 when the hairy pups were removed from the nest and sacrificed. Subsequent changes in body weight of the nude offspring were recorded.

Results

The change in body weight experienced by 3 nude pups following removal of the 4 hairy littermates is shown in Fig. 11. The mean daily weight increase from day 2 to removal of the hairy animals at day 6 was 0.18g/mouse/day, compared with 0.68g/mouse/day for nu/+ animals. Following removal of the hairy pups, a small increase in daily weight gain of nudes, to 0.23g/day occurred. However, by day 9, suckling difficulties were experienced and by day 12 one nude had died and the two surviving ones had experienced a reduction in body weight, equivalent to a mean value of 0.1g/mouse/day. On day 13 the second nude was dead and by the next day the last nude pup had died.

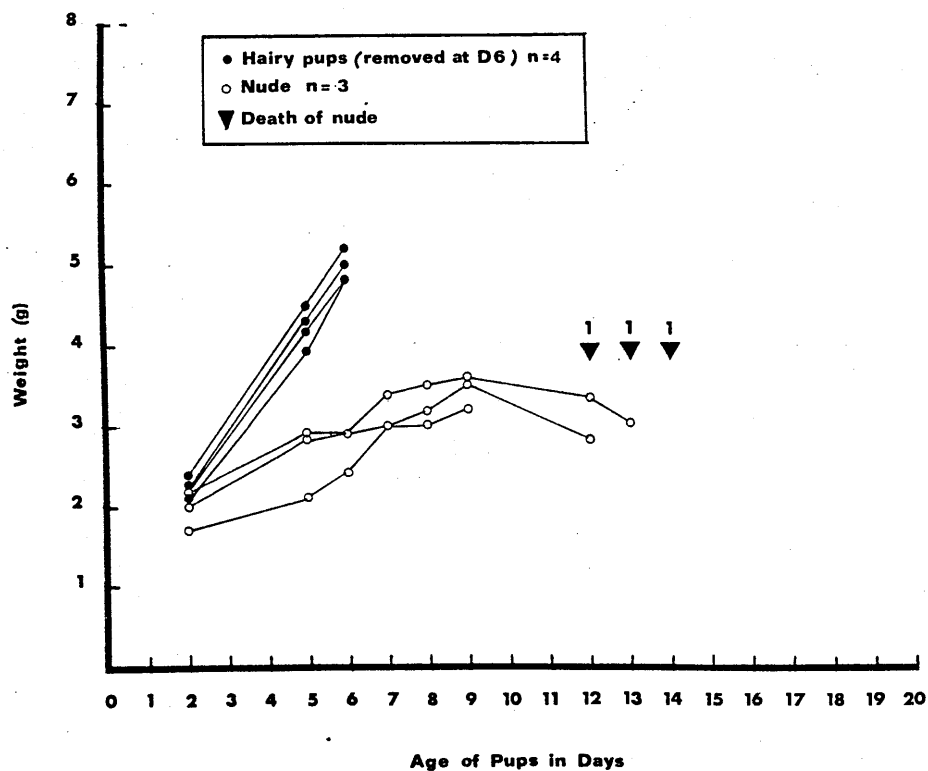


Fig. 11 Experiment 9: Removal of Hairy Pups at Day 6

Following removal of the hairy pups a small increase in daily weight gain for nudes occurred. On day 9 they weighed a mean weight of 3.5g which correlated with the standard curve (Fig. 9). At this point lactation/suckling failed and by day 14 all nudes were dead.

Discussion

In removing the competing hairy mice at the end of the first week of life, more milk should have been available for the nudes with concomitant opportunities to suckle.

All three nude pups did increase in body weight until day 9, following removal of the hairy pups, but only in line with the standard growth curve for nudes in mixed litters, and a tendency to the typical flattening-out of the curve was shown.

Re-examination of the pups after a further 3 days (without intervening examination or disturbance), showed that all pups had lost weight dramatically, and one pup had died, suggesting that lactation and/or feeding had been suppressed, coinciding with removal of the hairy sibs.

Lactating animals are normally good at adjusting milk supply to demand. In mice, over a range from four to twelve young, a threefold difference in demand, lactation is so regulated that weaning weights in individuals differ by not more than 10% on average (Findlay, 1974). If a dam has been nursing a large litter, she should soon accommodate to a smaller litter.

It appears therefore that by reducing the litter to three nude pups, lactation should have continued uninterrupted, with the pups benefiting from an extra supply of milk. As the reverse was found in this case, we can look to problems with the pups as being the more likely cause of feeding failure.

In the rat, negative (Sucking) pressure seems essential for successful feeding behaviour and removal of the salivary glands

prevents survival (Findlay, 1974). It is essential also, that the pups stimulate the mother to produce oxytocin and thereby enhance the release of milk during suckling.

It is likely therefore, that the nudes in this experiment were not able to obtain sufficient nutrient due either to deficient salivary glands affecting suckling abilities (Holmes and Mason, 1974) or to their inadequate stimulation of the mother, causing depression of milk ejection (Drewett and Trew, 1978).

Because of their varying individual suckling abilities a small number of nudes may not therefore match the maternal stimulation effects found for a similar number of hairy pups.

Experiment 10: Removal of hairy pups at day 13Materials and Methods

Two experiments were conducted when, using separate litters, hairy animals were removed at a much later date post-partum, i.e. day 13.

10(a) No manipulation occurred until day 5, when body weights were first recorded. On day 13 the hairy animals were removed from the nest and sacrificed. Nude pups continued to be weighed.

10(b) In this experiment weighings first commenced on day 11. At day 13 the hairy pups were removed from the home cage and placed in a separate box in isolation. Both groups of mice were weighed until day 15, when hairy pups were sacrificed. Regular weighings of the nude pups continued.

Experiment 10(a) Removal of hairy pups at day 13: with early handlingsResults

Up to day 13, the hairy animals had increased their body weight by a mean value of 0.44g/mouse/day, to an average weight of 6.3g per mouse. In comparison, the nude animals had only reached 3.5g, equivalent to a mean weight increase of only 0.19g/mouse/day, from birth.

The sudden increase in weight gain of nudes following removal of the hairy pups at day 13 was pronounced. Within 24 hours the nude animals had increased by a mean weight of 1.03g, which was five times greater than the previous increase.

However, this weight gain trend was not sustained. By the next day (day 15) a dramatic fall in body weight had commenced. The pups took on a dehydrated and weakened appearance and by day 18 one pup had died and the remaining animals weighed less than they did 5 days previously. The female was examined; the teats were not reddened or extended, suggesting that the pups had not recently taken milk. The female was gently restrained and individual pups were brought to the nipple. None of the pups were able to either locate nor attach to a nipple. On the next day (day 19) all 3 pups were found dead.

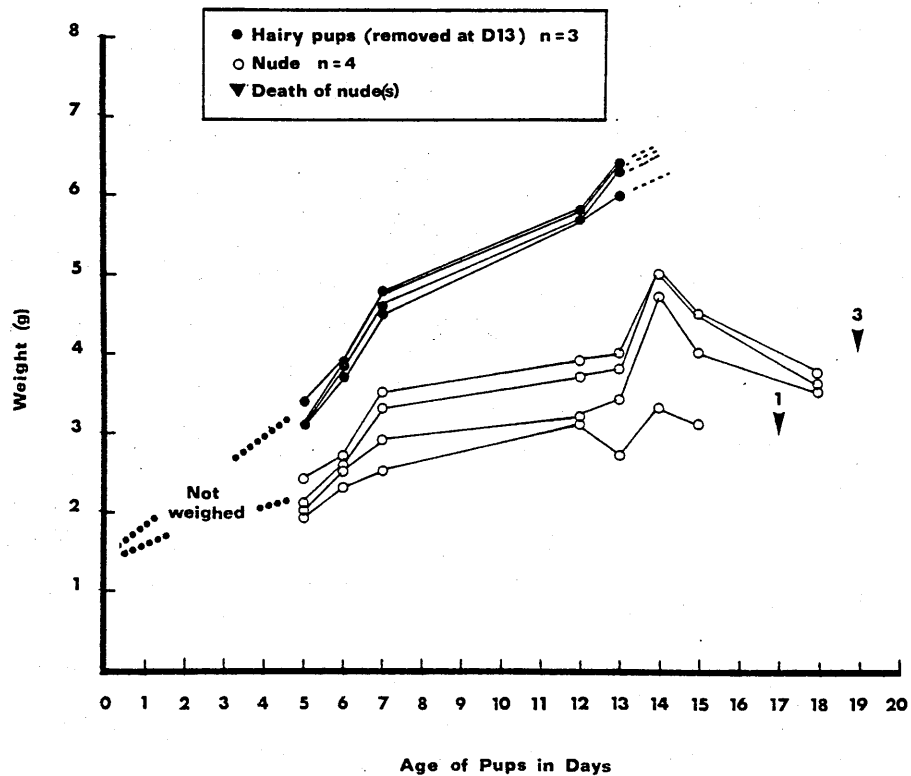


Fig. 12. Experiment 10(a) Removal of hairy pups at Day 13; with early handling (from day 5).

At day 5 all pups were first weighed, and subsequent weights were recorded, as shown. At day 13, the hairy pups ($n=3$) were removed and sacrificed and remaining nude pups ($n=4$) were left with the mother. Initially, the pups benefited from the absence of the hairy pups but within two days, lactation problems had developed and by day 19 all pups had died.

Experiment 10(b) Removal of hairy pups at day 13: without early
handling

Results

A litter similar to that described above was first handled and weighed on day 11. From day 11 - 12 both hairy and nude pups increased their body weight by a mean value of 0.5g/mouse, although the range for the nudes was much greater than for the hairy animals. On day 13 however all nude pups and 1 hairy pup experienced a fall in body weight. At this time the three hairy pups were removed from the nest and placed in a separate box. On day 14 the nude of lowest body weight was found dead but the other nudes had begun to gain weight. No other nude deaths occurred during the following two weeks and the mean weight increase was approximately 0.1g/day.

In the case of the hairy pups kept in standard conditions but without the mother a dramatic fall in body weight occurred with a mean loss of 0.43g on the first day and 0.75g loss by day 2 separation, when they were sacrificed, (day 15).

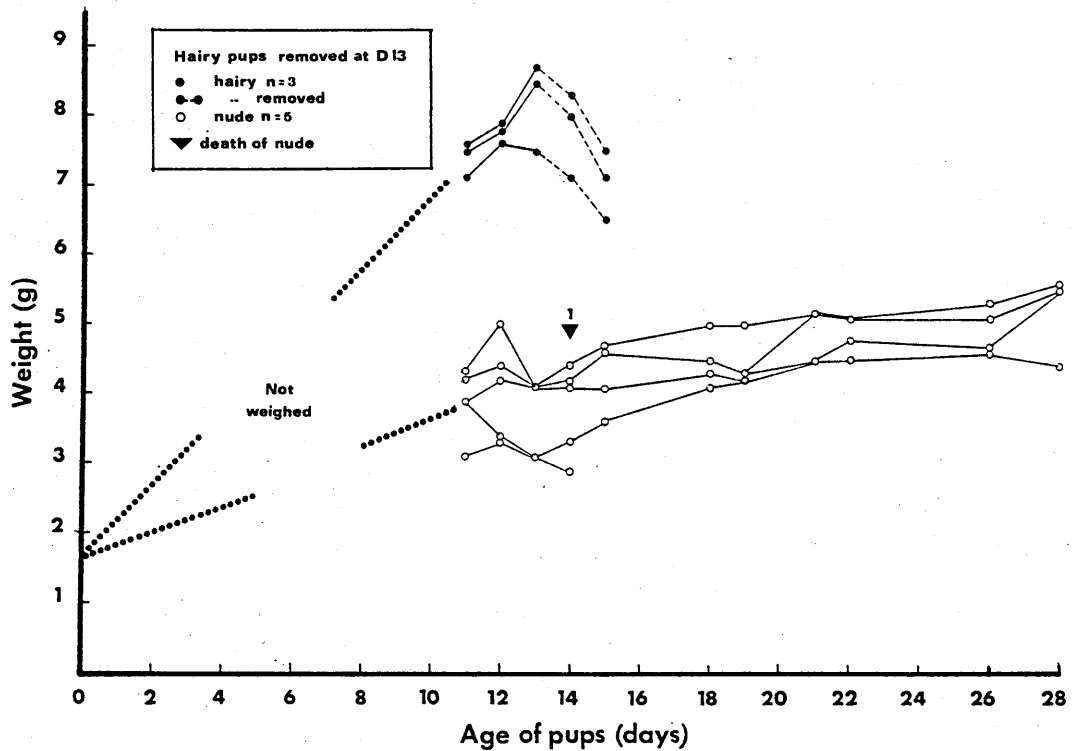


Fig. 13. Experiment 10(b) Removal of hairy pups at day 13; without early handling.

At day 11 all pups were first weighed and subsequent weights were recorded as shown. At day 13, the hairy pups ($n=3$) were removed and maintained in a separate box and sacrificed at day 15. Of the nude pups, 4 survived to weaning, when they had reached a mean weight of only 5g.

Discussion

In Experiment 10(a), the dramatic increase in weight gain within 24 hours of removal of all hairy pups from the litter suggests that the 13 day old nude pups were able to benefit from an increased supply of milk. However, the resulting progressive fall in nude pup body weight, with dehydration and weakness suggests a complete failure of nursing behaviour within 2 days of the removal of the actively suckling hairy pups.

As a rule, growth curves of mice reflect two breaks. The first at the end of the first week of lactation, and the second when the pups are about 2 weeks of age, when they begin to make a transition from milk to solid food; this correlates with opening of the eyes (McDowell et al., 1930).

Nude mice may be retarded in their development and thus at the time of the second break in growth described above, are unable to leave the nest and search for crumbs of solid food.

The mother, from day 12 - 13 is more prone to out-of-nest activity and will be less easily stimulated by pups into nursing activity, particularly if such pups display inadequate nipple search and attachment behaviour.

In Experiment 10(b), prior to removal of the hairy pups at day 13 both groups of mice were gaining weight at the rate of 0.5g/mouse/day. This, therefore was an example of relatively efficient suckling, particularly in the case of nude pups, although the differential weight between groups was preserved, i.e. the nudes weighed approximately 50% of the hairy pups. However, following removal of hairy pups the

suckling efficiency of the nude animals was greatly reduced and although viability of nudes was maintained, their mean body weight increase during the following 14 days was reduced to less than 0.1g/mouse/day. Their final body weight at weaning (day 28), was some 44% less than the standard curve for nude mice in the breeding colony. The very good level of nutrition obtained early post-partum (in the presence of hairy pups), obviously influenced their later successful development, although all pups experienced varying degrees of suckling success, as evidenced by the wide range of weights found at all ages.

The fall in body weight recorded for the three hairy pups in Experiment 10(b) were useful control values for the effects of known deprivation of maternal milk, and will be referred to in later experiments.

Experiment 11: Removal of some hairy pups (day 7);
returned to nest at day 10

Materials and Methods

At day 5 post-partum a litter consisting of 5 hairy pups and 2 nude pups was assigned to this experiment and body weights were recorded.

At day 7 three hairy pups were chosen at random and were removed to a separate box, supplied with nesting material only. Body weights were recorded for all mice.

After a separation of 3 days the hairy pups were returned to the home box (day 10). Regular weighings were made until death or weaning age was reached.

Results

The 2 hairy pups left in the nest with the mother experienced a growth rate consistent with good nutrition and at 28 days of age weighed 16g and 18g respectively, (within normal range). The shape of the curve resembled the standard curves shown previously, (Experiment 6)

Prior to their removal, the remaining three nu/+ pups displayed a similar weight gain, as was shown from day 5 - 7. In isolation, these pups experienced a fall in body weight and mean weight loss was approximately 0.5g/mouse/day, during the following 3 days.

At day 10 the hairy pups were returned to their home cage. During the next 3 days they returned to a normal feeding pattern and from day 13 their growth curve paralleled that of the hairy sibs kept with the mother. Results for the nude animals however, were quite different.

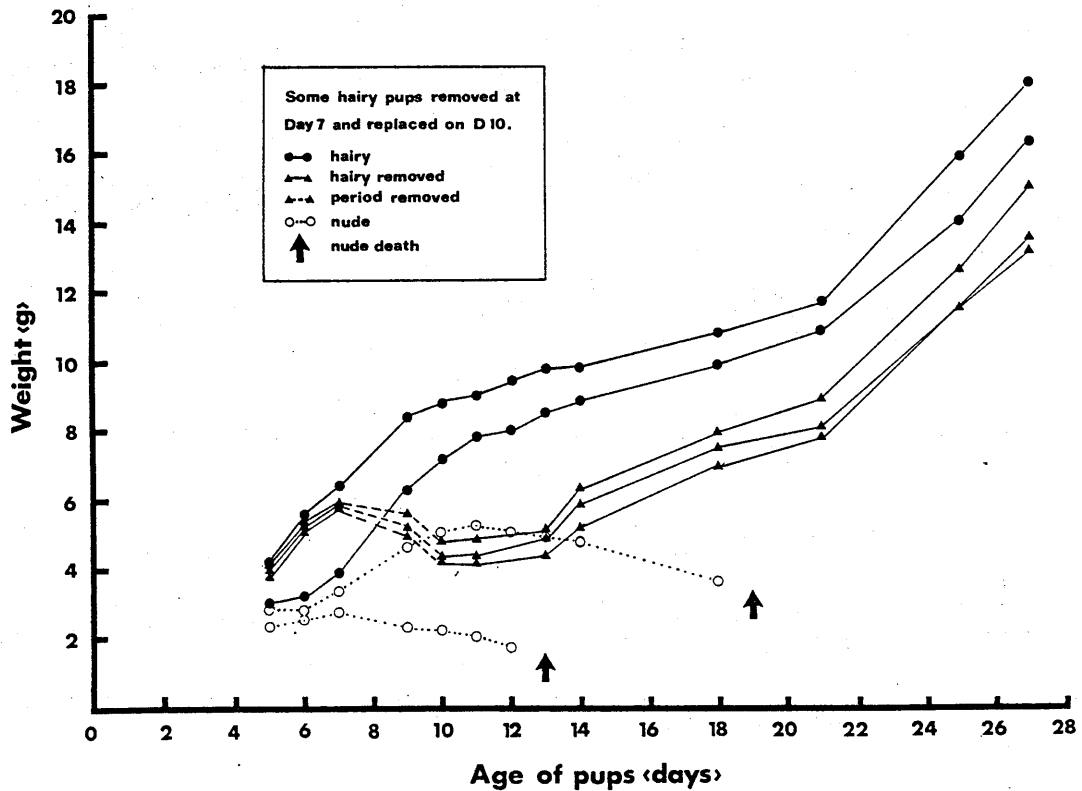


Fig. 14 Experiment 11: Removal of some hairy pups at Day 7; later returned to nest, (day 10)

At day seven, 3 of the 5 hairy pups were removed and placed in another box, with nesting material only. Body weights were recorded for all mice. After a 3 day separation the 3 pups were returned to the home nest and regular weighings were continued, until weaning. The two nude pups died before the fourth week of life.

● n = 2

▲ n = 3

○ n = 2

One nude was found dead on day 13 and the second nude (which had shown good physical development after removal of the hairy pups), began to lose weight when hairy pups were returned to the nest. This weight loss increased proportionately as the hairy pups developed until it also died on day 19. All hairy pups progressed well and at weaning (day 28) the deprived pups weighed only a few grams less than their peers.

Discussion

The results show that seven day old hairy pups are able to survive maternal deprivation for 3 days and then re-establish normal suckling behaviour after return to the home nest.

Small newborn mammals quickly develop hypoglycaemia if they are not fed; for example the blood glucose level of day-old rabbits which have not been fed is less than half that of fed animals of the same age (Hardman and Hull, 1969). Viability of hairy pups after a 3 day deprivation of food suggests that adequate energy stores are accumulated in the first week of life. They were certainly very able at re-adapting to the suckling situation and rapidly gained weight when returned to the litter.

It is significant that the more viable nude pup, left in the nest experienced the beginning of feeding problems on exactly the day that the 3 hairy pups were returned to the nest.

During their 7 day period of deprivation and 're-adaption', the three hairy mice did not increase their body weight above the weight achieved by the end of the first week of life and at day 14 weighed approximately 33% less than the control group. However, they all

weighed more than the most viable nude pup - which had remained in the home nest throughout.

It is significant that this nude pup began to experience a flattening of its growth curve, only when the hairy pups were returned to the home nest. Thereafter, it lost weight rapidly as the replaced hairy pups increased their weight, i.e. from day 13. This steady decline in body weight continued until its death at day 19.

During the third and fourth weeks of life body weight curves for the deprived hairy pups paralleled exactly those obtained for the non-deprived group and both groups displayed an accelerated growth curve from day 21 onwards, suggesting that the transition to an adult diet was progressing satisfactorily.

Experiment 12: Removal of some hairy pups at Day 13 -
later returned to home cage

Materials and Methods

In this litter, pups were not disturbed until day 10, when body weight for all animals was recorded; two hairy pups were selected for later separation. At day 13 the two hairy pups were removed to a separate box.

On day 16 these pups were returned to the nest. Body weights for all animals were recorded throughout this experiment until weaning (28 days).

Results

The results were very similar to those obtained in Experiment 10. The deprived hairy pups were able to suck with little difficulty and from day 18 their development paralleled that of the non-deprived group.

At weaning, their body weight was only a few grams less than the non-deprived group.

The nude pups were unable to maintain their body weight and on day 14 one pup was found dead. The remaining pup failed to develop and at day 17 began to lose weight; death occurred on day 21.

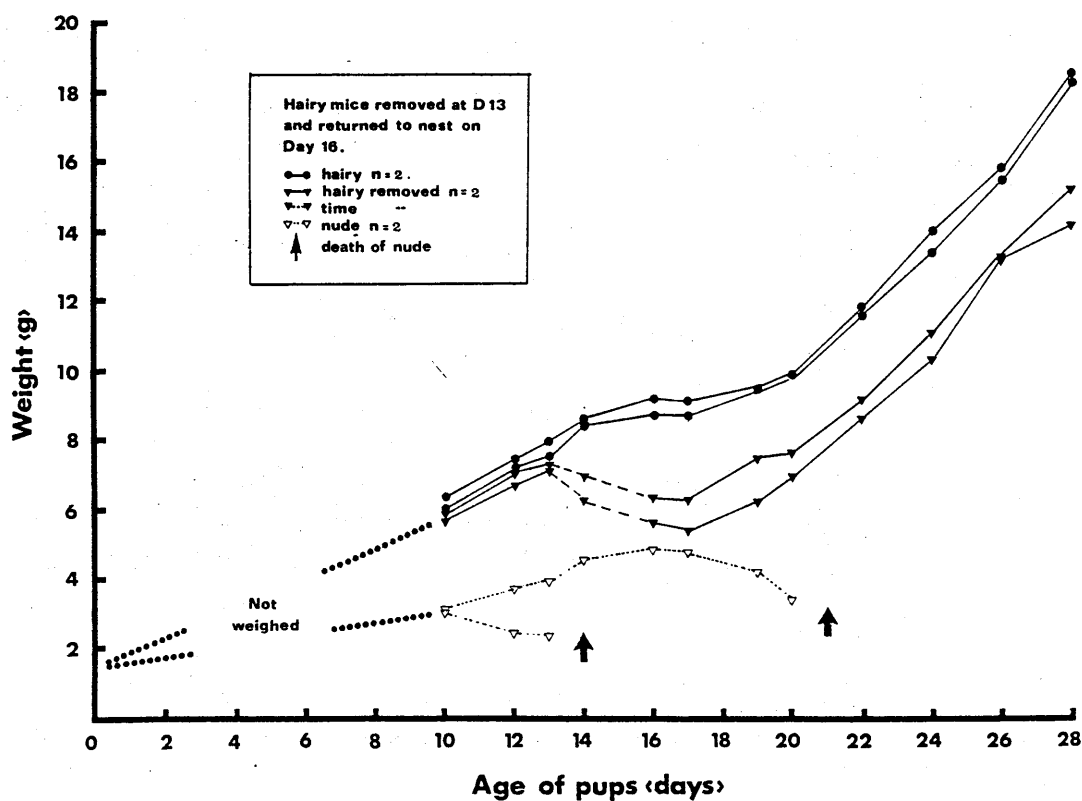


Fig. 15. Experiment 12: Removal of some hairy pups at day 13; later returned to nest.

At day 10 this litter was selected for study; pups had been subjected to normal maintenance regimes only.

Two hairy pups were nominated for removal at day 13. After a 3 day separation these pups were returned to the nest, where they developed successfully to weaning. The two nude pups died before the fourth week of life.

● n = 2

▲ n = 2

○ n = 2

Discussion

Heterozygous mice at day 13 were shown to be able to withstand a 3 day period deprived of maternal care and milk, without long term adverse effects on physical development.

Such hairy mice lost weight in a similar manner to the younger mice cited in experiment 11 but were more efficient at gaining weight during the 're-adaption period', upon return to the mother. This finding was possibly related to their greater degree of maturity and physical development.

From day 20 onwards, the rate of body weight increase paralleled that of the non-deprived group, although at weaning, the deprived pups weighed a few grams less than their littermates.

From day 10, one nude pup began to lose weight and was found dead on day 14. The remaining nude pup progressed at a standard rate and by day 16 had reached 5g in body weight, which fell within the expected range for age.

However, upon return of the hairy pups to the home nest on day 16, this remaining nude pup began immediately to experience nutritional problems as shown by the change in body weight.

During the next 3 days of life this nude pup lost weight progressively, at a similar rate to experimentally deprived animals, suggesting that the nude pup was no longer able to suckle efficiently due to competition from the full complement of hairy pups. From day 19 until its death on day 21 the rate of body weight loss was indicative of complete starvation.

In both experiment 11 and 12 the experience of the hairy pups shows that the mother was responding to stimulus from the litter, resulting in good milk intake for all hairy pups. This suggests that failure of the nudes in these cases again reflects inadequacies on the part of the nude pups alone, in finding, attaching to, or obtaining milk from the mammary gland.

Experiment 13: Ultrasonic vocalisation in nude and hairy pups

Materials and Methods

Facilities

The ultrasonic recording apparatus and laboratory facilities essential for this work were kindly made available at the Sub-department of Animal Behaviour, Cambridge by Dr S. Holman.

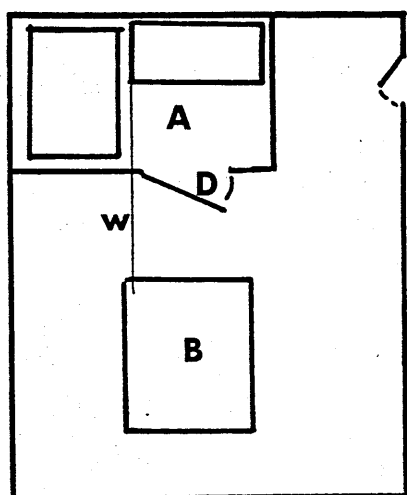
An outside laboratory used previously for similar work, was used with recording equipment in situ. The room was divided into two areas; one smaller area for housing the animals and a larger area where the bulk of the recording apparatus was positioned. A communicating door could be closed during the experiment to reduce background noise originating from the recorder motors.

Apparatus

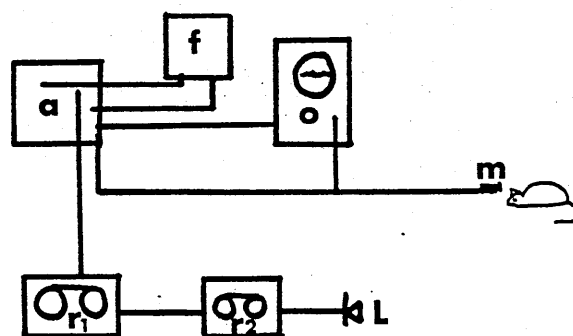
The recording apparatus was connected as shown in Fig. 16. The ultrasonic sensor (condenser microphone), filter, amplifier and oscilloscope were assembled in area A and a wire W connected this equipment to the recorders placed on table B. With the door D shut the background noise from the recorders was therefore kept to a minimum.

Animals

A standard breeding box containing eight pups, (4 nude and 4 nu/+) was transferred to the recording laboratory, where the temperature and lighting regime was kept the same as in the breeding room. The animals were allowed to re-adjust to this new environment for several days.



Ultrasonic test area



Recording apparatus

M	: Microphone
O	: Oscilloscope
F	: Filter
A	: Amplifier
R1 + R2	: Recorders
L	: Loudspeaker

Fig. 16. Ultrasonic Test Apparatus

The apparatus had the following characteristics:

Microphone: open circuit sensitivity at 1013 mbar was ± 48.7 db
or 3.67 mv per N/M^2 ; cartridge capacitance = 6.4 pf

Amplifier: meter range = 60 db/10 mv; range multiplier = ± 30 db

Filter range: 100 kHz

Oscilloscope: 100 mv/cm Y shift; Y shift coarse = 5 v/cm
variable time = 2 msec

Recorder: Lockheed; recorder speed = $33\frac{1}{4}$ i.p.s; playback = $1\frac{7}{8}$ i.p.s
and $1\frac{7}{8}$ i.p.s. onto a second sound recorder (Tanberg series
15 for sonagraph reproduction.

Recording technique

The microphone was positioned approximately 1-2 cm away from the pup's head. Ultrasounds were detected and displayed on the oscilloscope screen. Simultaneous recordings were made via the tape recorder, over a period of two minutes.

Play-back techniques

The two minute recordings were played-back at a slower speed, over 8 minutes using both tape recorders. The ultrasonic calls could be distinguished as heterodyne whistles and could be counted. Sonograms were produced from these tapes to provide a visual record of call frequency and time-scale. The final sonographs were kindly produced by Dr. P. Stolk on equipment at the Department of Clinical Veterinary Medicine, Cambridge.

Experimental Recordings

Day 5: As described previously (Noirot 1966), pups were chosen at random from the home cage and were placed alone in a small empty cage. A 2 minute recording was made and signals were monitored on the oscilloscope.

Day 10: The importance of cold stress and possibly hunger in eliciting isolation calls from infant rodents has been suggested by several authors. For this experiment it was decided to expose the pups to a very low ambient temperature, in isolation, whilst recording.

Each pup was placed in a metal box (approximately, 6 cm³ in volume) on a block of ice and allowed to cool for two minutes: a 2 minute recording was then made.

Day 17: The experiment was repeated under similar conditions to the above after an interval of one week.

Results

In the preliminary experiment on the litter at day 5, both groups of mice were able to produce high frequency calls which could be demonstrated as visual oscilloscope display and audio tape recordings. Two qualitative experiments were carried out in order to compare littermates.

From the experiment using 10 day old mice subjected to cold stress in isolation, results taken at random from the tape suggest that there may be a difference in calls produced by animals from different groups.

Nude pups produced a mean call rate of 38/minute, compared with 29/minute for hairy pups. The hairy pups produced a pulse of short duration, which started at about 128 KHz and rose to 144 KHz. The nude animals produced a call lasting approximately 120% longer than their hairy littermates (~~2~~ 308 m s), with a frequency range of 112 KHz - 136 KHz.

The same animals were re-tested at day 17. Only one animal appeared to produce ultrasonic calls (hairy pup) and this amounted to just 2 calls/minute. Essentially then, under the same isolation/cold stress conditions, the rate of calling had been reduced to almost zero for both groups.

Discussion

Using isolated Albino mice, Noirot (1966) found that relatively few high frequency calls were detected before 4 days of age. The

number of ultrasonic calls then appeared to rise suddenly on the fourth day of life, which coincides with opening of the ears. Practically no detections were made with pups older than 13 days, i.e. after the opening of the eyes. Many more calls were detected when pups were retrieved than when they were isolated.

The preliminary experiments in nude mice followed a similar pattern to those reported above. Both nude and hairy pups produced calls at day 5 and by day 17 (by which time eyes had opened in both groups) calling had been reduced to almost zero, as was found for P. maniculatus (Smith, 1972a).

The recordings at day 10 however, are interesting and may indicate that nudes respond to low temperature isolation at that age by producing a large number of calls of longer duration than hairy controls. Nude pups, lacking a covering of body hair are susceptible to hypothermia when isolated from the maternal nest and it would be essential for their survival, to be rapidly located and returned; older pups would be more able to thermoregulate.

If nude pups were experiencing suckling difficulties resulting in weakness and dehydration, it could be suggested that their ability to initiate and sustain ultrasonic calls would be impaired.

Such pups on the periphery or outside area of the nest would lose body heat, water and energy stores more quickly. This would lead to further weakness and possibly early neonatal death, if suckling was not effectively re-established.

Frequency

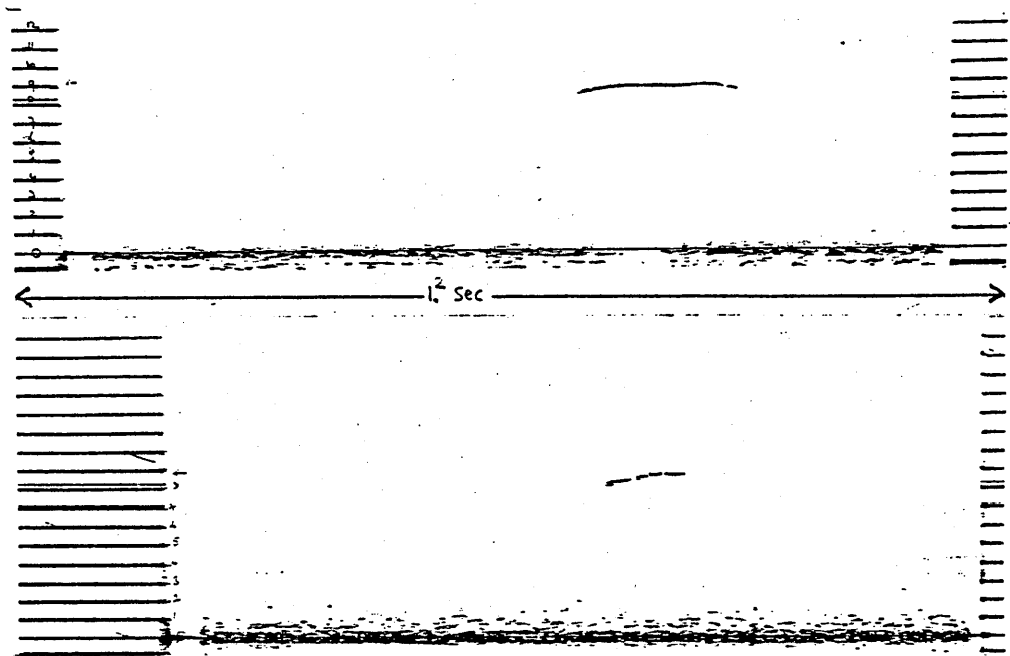


Fig. 18 Hairy call

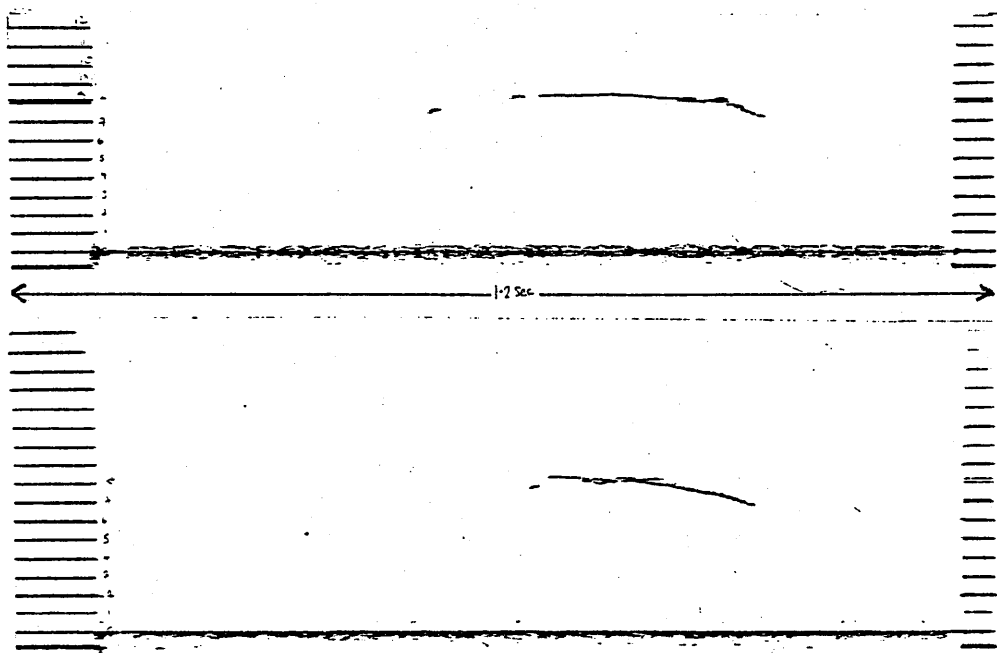


Fig. 17 Nude call

Time

Fig. 17 and 18 Ultrasonic calls in hairy and nude mice.

Eight 10 day old pups (4 nude and 4 hairy) from the same litter were subjected to low temperature isolation for 2 minutes and a 2 minute recording of ultrasonic vocalisations was made for each pup.

Two sonographs for each group are shown. The call produced by nude pups lasted approximately 308 ms ie. more than twice as long as the hairy pups, who had a mean call time of 140 ms.

Experiment 14: Development of locomotion in nude and hairy pups

Materials and Methods

Apparatus

A miniature open field apparatus measuring 30 x 30 cm with 10 cm sides was constructed from strong white cardboard. A piece of plasticised card (which could be easily cleaned) was marked into 5 cm squares with indelible marker and was fitted to the base of the apparatus.

Longitudinal study

A litter consisting of 3 nude and 3 hairy pups was selected, and animals were tested at 6, 9 and 11 days of age. Pups were taken at random and placed in the centre of the field. A stop-watch was started and the following data was recorded:

- i) time to first move from starting square
- ii) number of squares crossed in each minute of a 2 minute test.

The test was carried out in standard lighting and temperature conditions, as found in the breeding rooms.

Cross sectional study

A further four litters aged 6, 9, 11 and 12 days of age were assigned to this study and were tested as described above.

Results

Longitudinal study

The results shown in Fig. 20. indicate that nude animals were slow to begin ambulation at any age tested and at day six, 2 out of 3 animals did not move at all. On day 9 they were much quicker to commence movement but even by day 11 were still unable to achieve

scores approaching that of their hairy littermates. A Count of the total number of squares crossed showed that at all ages tested, nude mice were unable to score as high as nu/+ littermates.

The manner of movement displayed was also quite different between groups, with nude mice often 'freezing', and then moving in a distinctive 'circular' motion so that they did not readily move into an adjacent square. The hairy mice were more confident in their actions and were more likely to enter adjacent squares with swift, lateral movements.

Cross Sectional Study

A study of larger numbers of animals who were tested once only, gave similar results to those above. At all ages tested, hairy animals were more likely to move than nude littermates.

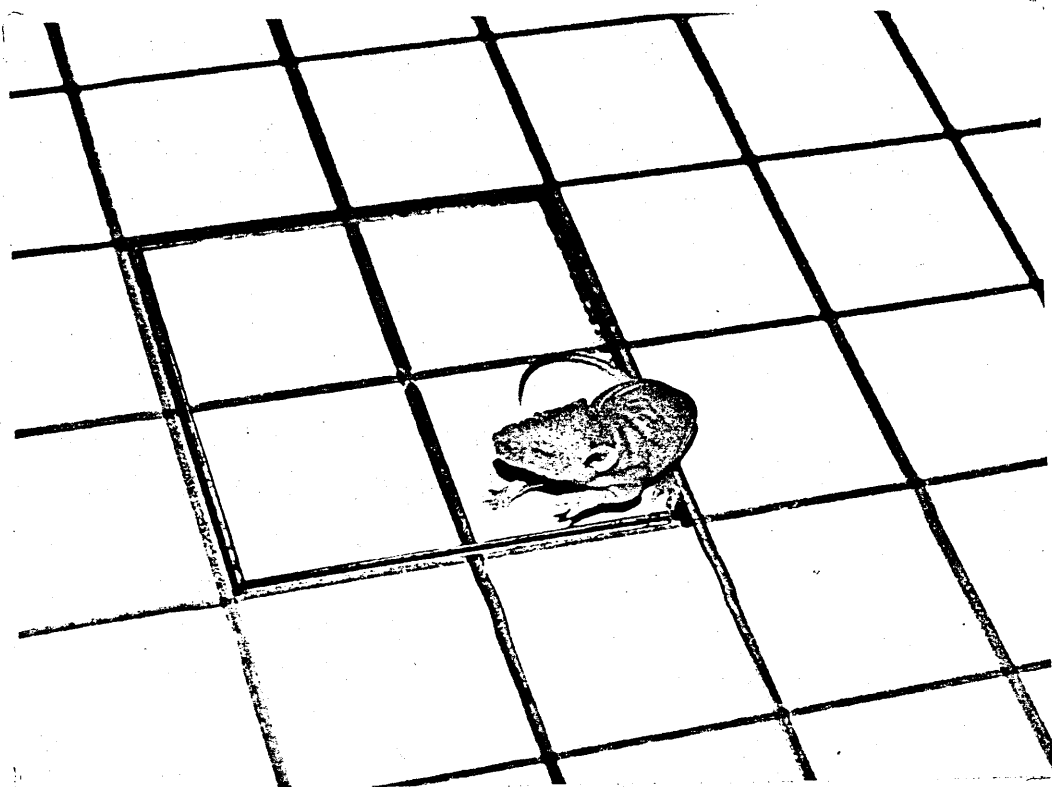


Fig. 19. Test for development of Locomotion

A miniature open field measuring 30 x 30 cm was marked out into 5 cm areas; mixed litters were tested at 6, 9 and 11 days of age. The nude mouse above aged 6 days, displays the typical circular motion which resulted in low scores, as the mice did not readily move into adjacent squares.

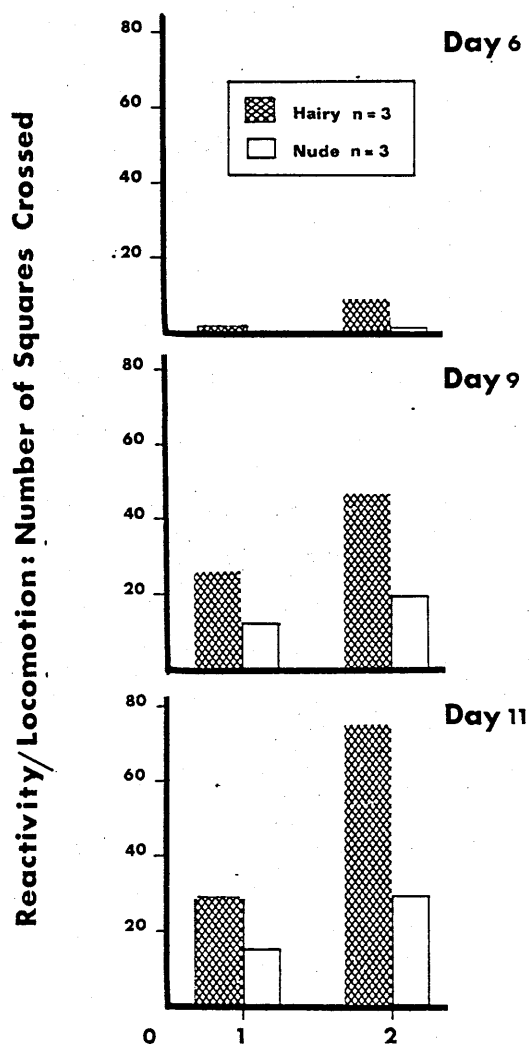


Fig. 20. Longitudinal Locomotion study on one litter

A single litter consisting of 3 nude and three hairy mice was tested on three occasions at 6, 9 and 11 days of age. The histogram illustrates the number of squares crossed in the miniature open field for each minute of the test.

Age	Group	Time to 1st move (secs)	No. squares crossed		Total
			1 min.	2 min.	
6 days	hairy (n=5)	28.5	3.8	9.6	11.4
	nude (n=5)	66	0.2	1.8	2
9 days	hairy (n=3)	14.6	9.3	7.3	16.6
	nude (n=3)	20.3	6.5	3.6	10.1
11 days	hairy (n=2)	9	19.5	18.5	38
	nude (n=5)	34.4	8.5	5.3	13.8
12 days	hairy (n=4)	7.7	31	37.8	68.8
	nude (n=4)	14.2	9	15	24

Table 9. Development of Locomotion

Hairy and nude mice from the same five litters were tested once only at the ages shown. The first result shows the time taken to make the first move from the starting square and the last figure shows the total number of squares crossed in two minutes of testing.

hairy n = 18

nude n = 21

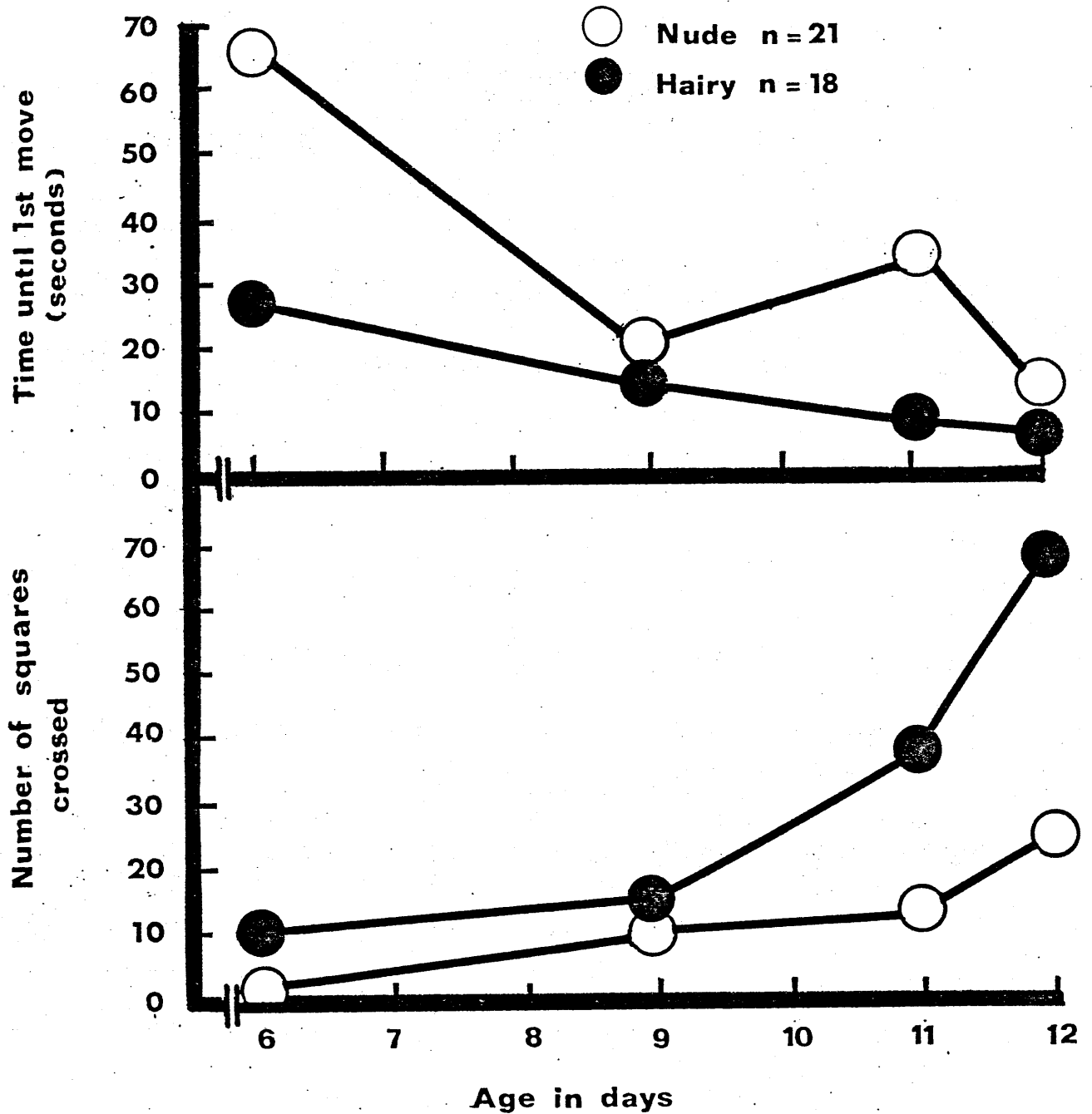


Fig. 21. Development of Locomotion in Nude and Hairy Mice: Cross sectional study.

Mice from four litters were tested in a miniature open field for 2 minutes, once only at 6, 9, 11 and 12 days of age. At day 6 hairy pups were active in the open field and were beginning to move in a co-ordinated pattern. By day 9 however, nudes were still moving in a primitive circular manner resulting in low scores. Results on day 12 showed that nudes were nearly 3 days behind hairy pups in locomotion development.

Discussion

The study of behavioural development in the mouse by Fox (1965) showed that during the period day 3 - 9, strong stereotyped reflexes and limb placing reactions occurred, with pivoting and circular locomotion behaviour. During the day 9 - 15 period (postnatal transition period) adult locomotor activities appeared.

Table 9 shows that at day 6 hairy pups were very reactive in an open field and were able to move in a more co-ordinated pattern than nude pups of the same age.

By day 9 nude pups were still moving in a primitive circular manner, using pivoting and crawling locomotor patterns. Hairy pups were beginning to move in an adult-like manner and by day 11, were achieving high open field scores.

Testing on day 12 showed that nudes were nearly 3 days behind hairy littermates in refinement of locomotor abilities and by the end of a 2 minute test had reached little more than 34% of the score achieved by hairy pups.

Fox had studied several runts whom were physically smaller than their littermates. Such mice had retarded hair growth, eyes opened late (15-17 days of age) and had weak body righting and locomotion behaviour; poor responses at 18 days of age indicated that muscular weakness was coupled with delayed development of adult behaviour patterns.

The results for nude mice suggest that nudes may display retarded development as measured by the development of locomotion behaviour which is associated with late eye opening and possibly general body weakness, and may point to a nutritional influence.

Experiment 15: Exploratory Behaviour at Weaning

Materials and Methods

Apparatus

An apparatus similar to that previously described by Thompson (1956) was constructed from $\frac{3}{4}$ " thick plywood. A square base measuring 30" x 30" was divided into 5" smaller squares with 12" boundary walls as shown in Fig. 22.

At the base of each alternate smaller square, a single unit barrier measuring 5" x 5" was placed; there being 15 of these in all.

The apparatus was coated in clear varnish to waterproof the material and facilitate easy cleaning after each test.

Test procedure

Litters consisting of both hairy and nude mice were transferred from the breeding room to another barrier room at weaning, (28 - 30 days of age). Animals were then tested in the arena within 7 - 10 days, under standard temperature and lighting conditions.

Each mouse, nude or hairy, was chosen at random, placed in the starting area and given 5 minutes to explore the enclosure. A record was made of the number of lines traversed per minute of observation.

Following the test the animals were returned to their home cage and the apparatus was cleaned and air-dried, prior to the next litter being tested.

Ten litters from the standard breeding room were used for the 'Thompson Test' (1956), consisting of 27 hairy mice and 46 nude mice (total = 73), aged between four and five weeks of age. The animals had experienced normal environment/development and had not been manipulated in any other experiments prior to testing.

Results

The hairy mice were positive in their actions and quickly moved from the starting square. They reached an activity peak at 3 minutes and would often traverse several times across the arena. They seldom 'froze' or lingered in a square, indeed on many occasions it was difficult to keep count of such active movements. Two mice actually attempted to jump over the 5" barriers. Activity levels tended to equilibrate towards the end of the test.

Nude littermates invariably gave distinctly different results, shown in Fig. 23. The slope of the activity curve shows that the nude mice lacked confidence as evidenced by the slow increase in activity levels which only just peaked at the end of the test. This group were slow to commence movement and often returned to the starting square and paused before exploring a different area. Their movement across the arena was deliberate and hesitant and some mice would peer around barriers for some time before moving into the next square. A few mice only, demonstrated high activity levels throughout the test.

Few animals from either group were observed sitting or lying for any length of time and grooming actions were uncommon. Most of the animals passed some urine or faeces and although some appeared to exhibit this in a possibly 'emotional' manner this aspect of the test was not evaluated.

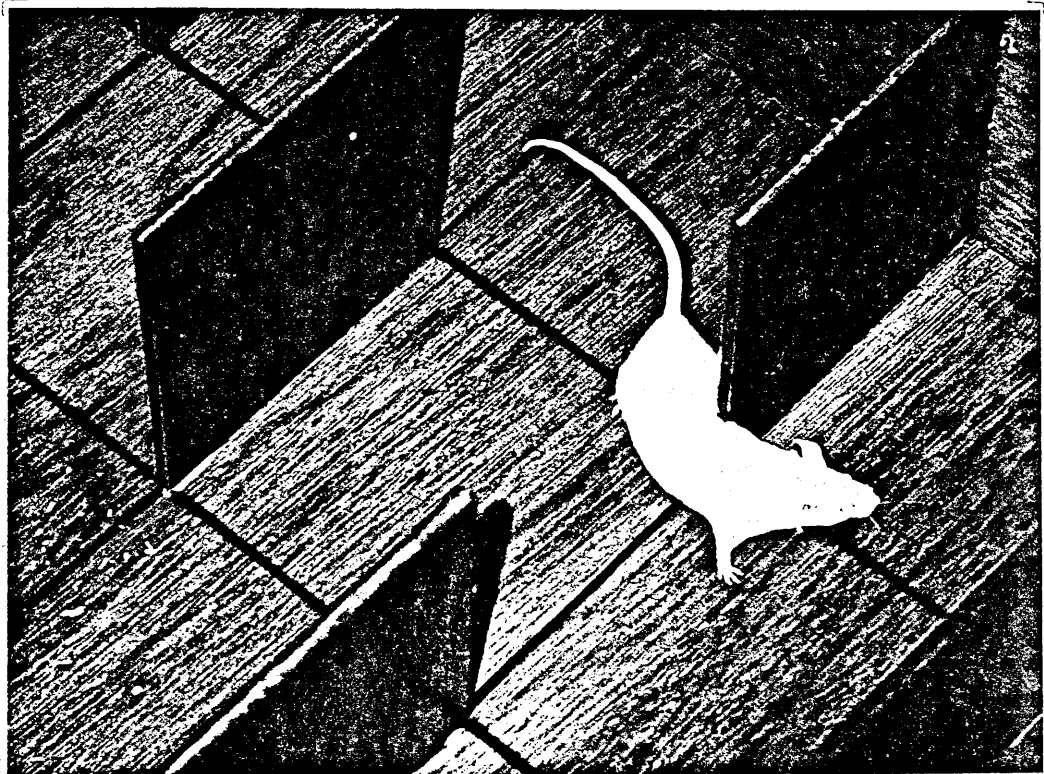


Fig. 22. Exploratory Behaviour Test Apparatus

The apparatus was similar to that described by Thompson (1956), constructed from plywood and measured 30" x 30", divided into 5" squares with 12" boundary walls. At the base of each alternate square a single unit barrier (5" x 5") was positioned, (total of 15).

Weaned hairy and nude animals were tested for 5 minutes and the number of lines crossed and the patterns of behaviour demonstrated were recorded.

Group	Number	Mean Number Lines Crossed	\pm S. D.
Hairy	27	132.55	70.12
Nude	46	70.71	37.96

(i) Exploratory Behaviour: total number of lines crossed in 5 minutes for 10 litters; ($P < 0.001$)
(student 't' Test)

Group	Number	Number of Lines Crossed / Minute				
		1	2	3	4	5
Hairy	27	17.55	26.11	29.48	28.48	30.48
Nude	46	8.19	13.11	14.83	16.11	19.39

(ii) Exploratory Behaviour: Activity/minute during the 5 minute test for 10 litters.

Table 10. Exploratory Behaviour in weaned animals

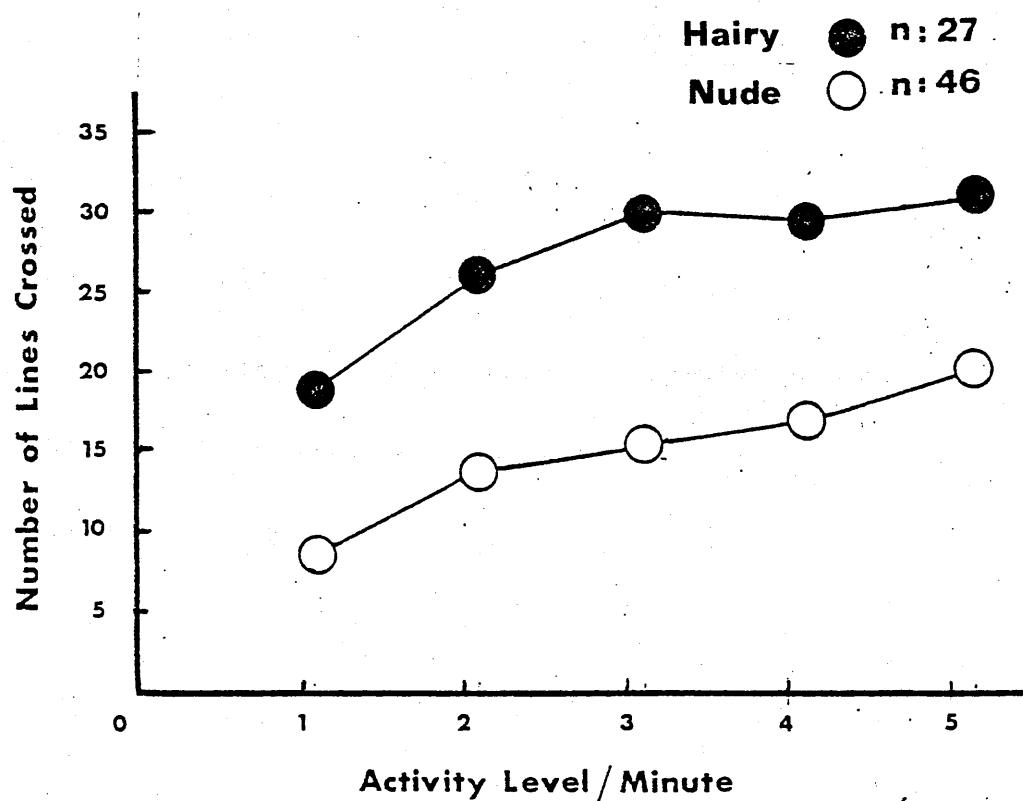


Fig. 23. Exploratory Behaviour in weaned animals

Hairy mice achieved higher scores than nude littermates. The variance of groups means over the total test was significant ($P < 0.001$)

Discussion

In Thompson's experiments 15 strains of mice were tested in an Open field and each strain gave a different measure of exploratory activity, which ranged from a score of 20 squares crossed for A/CI mice to 459 squares for C57BR/a mice over a 10 minute test period.

The results for the nude mice study show two major differences between groups, over the 5 minute test period.

Firstly, the total number of lines crossed was quite different. The hairy mice obtained a mean score of 132.55 (S.D. \pm 70.1) and nudes scored 70.71 (S.D. \pm 37.9). In addition, the manner in which the animals moved was different on purely descriptive grounds.

Hairy mice showed a strong inclination to cross the test field. They were not deterred by the presence of barriers, they did not 'freeze', nor spend time in other behaviour activity other than locomotion. The rate of ambulation per minute increased steadily to a maximum score after 3 minutes of testing. Thereafter scores remained high but did not increase further.

From the start of the test, nude animals were very slow to move; after moving into an adjacent square they would often hesitate and then return to the starting square, taking-up a position next to a barrier.

Nudes appeared to exhibit an 'emotional' or timid state, were liable to 'freeze' and never attempted to leap over a barrier, as found in the case of some hairy mice. A study of their activity level/minute shows that nudes were about half as active as hairy pups up to

three minutes of testing. Scores for nudes continued to rise during the remaining 2 minutes of the test, suggesting that they may have been adapting to the novel situation.

If it is valid to draw a correlation between ambulation and exploratory behaviour, it may be assumed that low ambulation indicates high emotionality, resulting in animals which 'freeze' when exposed to novel environments. Archer (1973) however, in a comprehensive review, cites evidence which shows that such tests are far from being unequivocal.

As both groups of mice have the same genetic background, it can be suggested that the significant difference in locomotion and exploratory drive may result from either the effects of the recessive mutation, (affecting hair growth and thymus development) or changes in behaviour due to postnatal development or nutrition. The reports of Denenberg et al., (1963) using hairless mutant mice (hr/hr) with intact thymus, showed that such animals were also significantly less active than controls in the open field test.

The connection here then, is the absence of hair and is useful evidence that the effects of thymic aplasia having perhaps, some effect upon the endocrine system, may be disregarded. The explanation therefore probably relates more specifically to the effects of differences in nutrition and early experience.

GENERAL DISCUSSION

Using barrier maintenance conditions nude mice were successfully produced during a five year period. Reproductive activities were satisfactory and compare well with previous reports (Festing and King, 1974).

The incidence of infection or 'wasting disease' in the general breeding rooms was low and reflected the very high standard of cleanliness and care provided by maintenance staff.

The average nest temperature for an animal house maintained at 21°C has been determined as 33°C (Porter and Festing, 1969). As the rooms in this study were kept at 27°C, it is probable that nudes were kept adequately warm, both in and out of the nest (Rygaard, 1973).

Throughout the behavioural and developmental tests animals were maintained in conditions used for standard husbandry and maintenance and the nude male parent remained with the lactating female mouse and pups.

A small number of nu/nu x nu/nu homozygous matings were attempted. Six litters resulted but within 48 hours all offspring had died. It appeared that female nudes were not interested in normal maternal behaviour, made little attempt to maintain an intact and warm nest, moved continually around the cage and prevented the young from adopting successful suckling positions. Festing and King (1974) noted that female nudes were unable to rear their young, although Ohsawa et al., (1974) showed that the endocrine functions of nude mice were similar to BALB/C controls.

Nest building and related activities are an important measure of maternal behaviour development (Noirot, 1966). Lynch and Possidente (1978) found that the amount of cotton wool used for nesting increased soon after mating and remained high with a large amount of variability until about 10 days after parturition - then decreased sharply.

Heterozygous nude mice appeared to produce excellent nests for the first week post-partum and adequate nests until the pups were about two - three weeks of age. By day 21 however, nests were becoming poor, flat and offered little protection or thermal insulation.

It is possible that nude and hairless litters, although normally containing some hairy sibs, may not be maintained always at optimum temperature. In such conditions, particularly with a prevailing low ambient temperature, dams need to build large nests. Both huddling and nesting however, can interact to reduce heat loss, resulting in small nests (Lynch and Possidente, 1978).

This study shows that a particular nest building pattern may be followed including construction of the nest at a specific site, i.e. near-to or under the overhanging food/water containers. Whether this site is chosen for reasons of protection or convenience is uncertain, but the results of Nesting Experiment 1, suggest that the female mouse will move her litter and nest to maintain the status quo. A study of two more litters showed that the female parent would build and maintain the nest near (to) the food supplies. Similar results have been reported for the hairless (hr/hr) mouse, (Thatcher, 1980 personal communication).

Observations by McCarty and Southwick (1977) in gerbil litters where the male was present, showed that pups were rarely left unattended in the nest by both parents simultaneously. The female may however, aggressively exclude her male partner from the nest area during the first few days after parturition; gradually the male was allowed entry to the nest and by day 5 was fully accepted.

The present nude mouse study showed that pups were given similar treatment to that above, with one or both parents always in the nest. Both parents retrieved and returned displaced pups and helped to maintain the nest. In these studies however, the male spent most of the observation time in the nest during the first week post-partum and the threatening postures and vocalisation displayed by the female, described at this time by McCarty and Southwick, were not manifest. It was apparent that in most cases the male was as 'maternal' as the gerbil female (Elwood, 1975).

In many species for example cat, rat and rabbit, the earliest nursing - suckling episodes appear to be initiated by the mother, who may stimulate the neonates to suckle by licking them, before presenting her mammary surface to them so that the minimum of exploratory activity is needed for the offspring to locate the nipple (Findlay, 1974). Without nipple attachment and stimulation from the pups however, efficient suckling cannot occur.

During the perinatal period the nude and hairy mouse pups spend most of their time with the mother, sleeping or nursing. After a few days post-partum the actual position of the pups relative to the mother's nipples could sometimes be ascertained. The general results show that in the case of nude animals the actual time spent in nipple

contact may be very restricted. It is apparent that nude pups have difficulty in finding and attaching to the nipple and may lack strength to enable them to maintain hold, when the mother moves or when pushed aside by hairy littermates.

Suckling in the blind and deaf newborn rodent is controlled by a narrow spectrum of stimuli - olfactory, thermal and tactile - that define the mother and more particularly her mammary area. The nu/+ females used in this study invariably develop active mammary glands and encouraged suckling, as evidenced by observations on nursing behaviour and growth curves of normal offspring.

As the nude pups are deficient in whiskers it may be suggested that the 'vibrissæ placing response' (Fox, 1965) which enables them to make contact with solid objects is depressed. Alternatively, the 'chemical cue' suggested by Teicher and Blass (1976) as essential for suckling may be absent. These authors propound that normal suckling requires the 'cue' to be present on the mother's nipples and may be secreted by her and more importantly by the pup itself. Of course such a mechanism could only function if the olfactory organs and salivary glands of nude mice respond adequately and there is no evidence that this is the case either. In one litter studied, a nude pup was observed attempting to suckle from the male parent - no hairy pup displayed any similar action.

The fact that nude mice in mixed litters progress for the first few days of life satisfactorily is interesting and suggests that normal suckling may be occurring at this time. Teicher and Blass (1976) agree that initial suckling is probably not influenced by a chemical cue from the (rat) pup but is more likely to result from maternal influences.

Early attachment may be the result of a pheromone secreted by the nipple itself, or the mother's saliva, or birth fluids which are spread appropriately by the mother. Alternately, the first attachments are not under olfactory control (Teicher and Blass, 1976).

Recent work (Moore, 1981) has demonstrated further the great importance of olfactory cue detection and showed that olfactory stimuli from rat pups both stimulate maternal licking and serve as a method for discrimination of pups on the basis of sex. Smotherman et al. (1978) report that pup odour, whilst not serving as a directional cue, determines the speed at which the female initiates her search behaviour and encourages her tendency to approach ultrasounds.

In the study by Kovach and Kling (1967) using kittens, there was a definite cut-off period, after which it was impossible for animals deprived of the opportunity to suck to later initiate suckling. Such animals displayed the rooting reflex upon stimulation of the facial areas but they were not able to locate and respond to the mother's nipple.

If deprived kittens were forced to grasp the nipple by an experimenter, no sucking reflex was noted and nipple hold was soon lost. The insertion of an artificial teat into the animal's mouth did however, result in milk flow, supporting the suggestion that it is not the sucking reflex but the ability to locate and respond to the mother's nipple that was absent in these animals; a similar situation may be occurring in the case of some nude mouse pups.

In the above experiments, the mother responded to all deprived kittens, either immediately or after a short delay by licking them vigorously and by assuming the characteristic outstretched nursing position; heterozygous nude mouse mothers in the present study responded to nude offspring in a similar manner, even when nude pups failed to find the nipple during a 15 minute observation period.

The preliminary experiment on ultrasonic calling in nude mice described in Experiment 13 suggests that homozygous nude mice may produce longer calls of greater rate and frequency than in the case of hairy sibs, under similar test conditions. The results at day 10 suggest that the nude offspring were more susceptible to cold stress than hairy pups, although able to tolerate similar stress at day 17. Okon (1970a) found that the rates of calling at this age increased from 4 calls to 32 per minute ($^{\circ}/_f$ 38 for nudes) and that animals aged 10-11 days maintained at low temperature, call more intensely than control groups at ambient temperature of 33°C .

If nude mice respond to isolation/cold stress by producing more intense ultrasonic calls, then this would explain why the parent is more likely to retrieve nude pups first. Nude mice would often receive intense licking actions from the mother, suggesting further, the influence of ultrasonic calls (Noirot, 1969a).

When the ultrasonic test was repeated at day 17 the incidence of calling was almost reduced to nil for both groups. As retrieval of nude pups beyond this age still occurred, presumably the visual stimuli of 'immature forms' was sufficient to induce the parent to retrieve. Alternatively, the presence of 'pup odour' causes her to initiate search behaviour (Smotherman, 1978) and may also enable her to discriminate between pups (Moore, 1981).

Some studies with rodents have shown that increasing litter size permanently reduces body growth and conversely, reduction of litter size enhances growth and development. Such alterations in litter size have also been shown to affect adult behaviour with resulting effects on pup development; for example, behaviour differences may be due to alterations in maternal behaviour, as rats and mice nursing small litters spend more time in the nest with their pups and more time handling them.

In recent experiments, Teicher and Kenny (1978) made the important observation that reducing litter size has the surprising effect of reducing both the willingness of pups to suckle and the vigour of their search behaviour. Moreover, these effects seemed more directly linked to alterations in the litter experience than to nutritional differences.

They reported that by 10 days of age serious defects in suckling behaviour were noted in pups raised in small litters, i.e. 3 pups. Testing such pups on a mother raising large litters with an average of 12 pups, had absolutely no restorative effects on suckling. This indicates that the deficit is probably primary to the pups and is not simply due to some inadequacy of their mothers.

Other experiments however, showed that the suckling behaviour of 5 day old pups was less markedly affected by alteration in litter size but that the raising of pups in litters of three markedly affected pre-weaning suckling by reducing the vigour of their search behaviour.

MacDowell et al. (1930), investigated the effect upon bodyweight in suckling mice of reducing the number of pups per litter, thereby increasing the availability of maternal milk. They compared the published weights for the suckling mouse, obtained from twelve

different literature sources - the upper and lower limits of these curves are shown in Figure 24.

Considering the great range in material, methods and laboratories these curves were fairly consistent. Birth weights were similar and at 14 days the range in weight was 4 - 7 g. by the third week the range had extended to 5 - 11 g. and at weaning (28 days), mice weighed between 7 and 15g. MacDowell et al's standard curve fell near the mean for the range but following reduction in pup numbers, weight gain was greatly increased. They concluded that reduction of the litter to four at birth, to two when three days old and to one when 5 days of age produced maximum growth.

In studying rat pups from birth until 16 days of age, Schultze (1954), showed that with small litters, e.g. 6 pups, the mother was not induced to maximum milk production. By increasing the litter size there was a progressive decrease in the mean weight increase of each pup but a marked increase in the total weight gain of the litter, reflecting greater milk production of the mother.

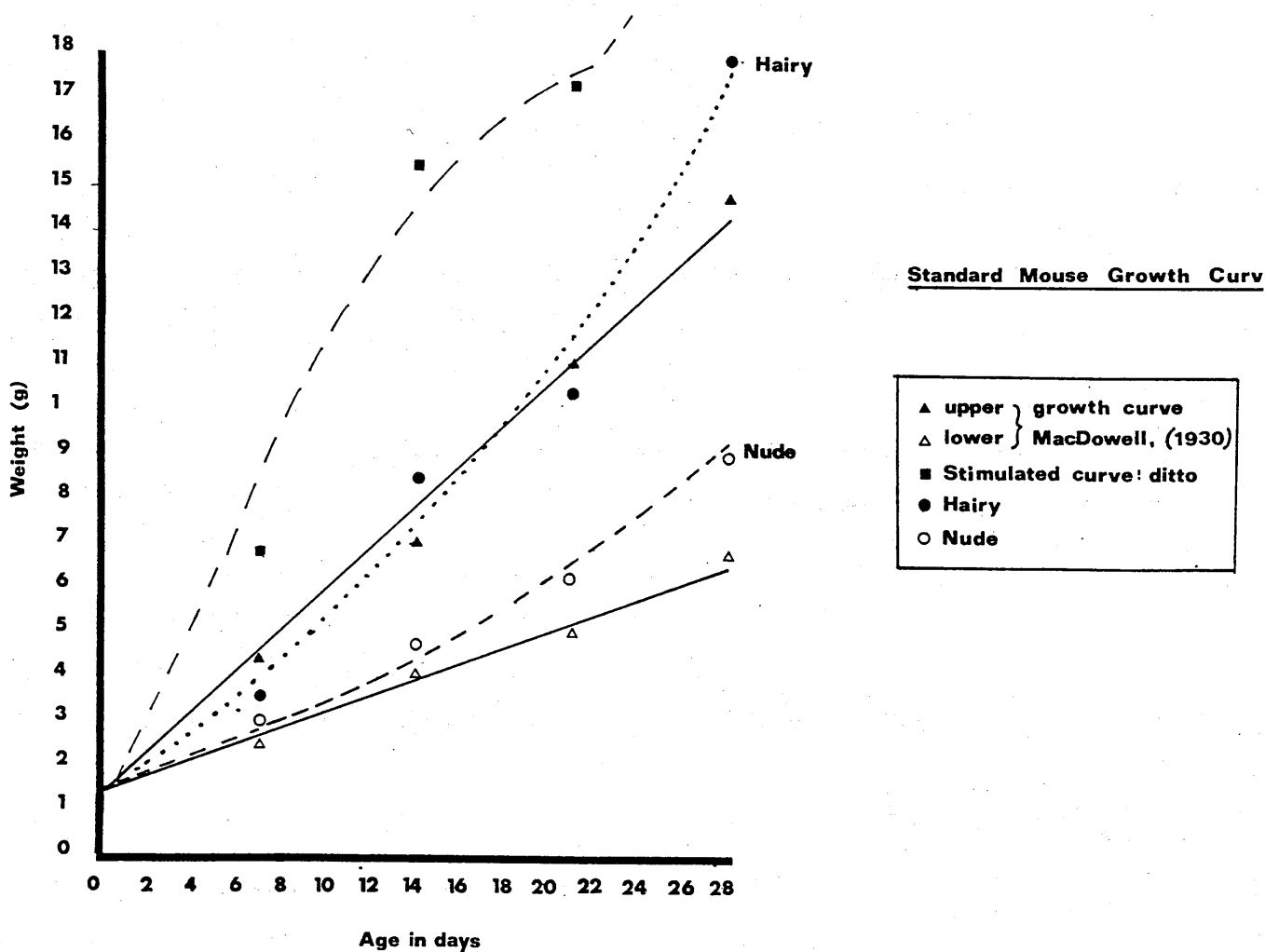


Fig. 24. Standard Growth Curves for Mice
(From MacDowell et al., 1930)

The upper (▲) and lower (△) ranges for body weight curves from 12 different literature sources cited are shown. The standard growth curves for hairy and nude mice in this present study are plotted against the above ranges. The stimulated curve resulted from litter manipulation (reduction) experiments conducted by MacDowell et al and are described in the text.

(Lines were fitted to the points by eye).

Observations on the normal growth curve for mice in this present study showed that both hairy and nude pups had similar body weights at birth and gained weight in a similar manner until about 8 - 10 days of age. Thereafter, the rate and degree of weight gain for hairy pups was much greater than for nudes and at weaning, hairy pups weighed on average, twice as much as nude sibs.

This result was the same, both in a cross-sectional study of 100 animals and in a smaller longitudinal study of 3 litters. In almost all husbandry reports for athymic nude mice a differential trend in weight gain for groups has been observed.

A study of four mixed litters by Rygaard (1973) showed that nudes suffered a marked retardation of growth, which started on about the 13th day post-partum. Growth did not accelerate again until about day 21; this compares with day 12 and day 19 respectively for this present study.

When the results from this present study were plotted against the mean growth curves cited by MacDowell et al. (1930) for 12 mouse strains, it was found that the hairy pups closely followed the strain displaying the highest growth curve, whilst the nudes (from related litters), followed the lower curve. Thus in terms of weight-gain alone, these animals behaved as if they had developed from two different strains of mice.

MacDowell's work on stimulated growth, using litter reduction techniques is interesting but contrasts with the reports of Teicher and Kenny (1978). A study of the litter reduction experiments for nude mice adds further data for discussion.

Removal of all nude pups from a standard litter of mice has no untoward effect upon the subsequent development of the remaining hairy mice. Such animals gain weight in a similar manner to those left in intact litters.

When hairy pups are removed, the above result may not occur for nude animals. If all hairy pups are removed either immediately following birth or at various stages after birth, then generally the effects are detrimental. The nude pups begin to lose body weight, become weak and often die. It would appear then, that merely the presence of hairy mice in the litter is essential in many cases for normal development of nude mice. This finding supports the general literature agreement that in homozygous litters, where hairy pups are absent, failure to thrive and early perinatal death can be demonstrated. This result however, contrasts with Rygaard (1973) who showed that in 3 litters studied, nudes benefited significantly by removal of all hairy pups at day 0.

This present study shows that where hairy animals had been allowed to remain in the maternal nest since birth, the beneficial effects were clearly displayed. Subsequently however, whether the hairy pups were removed at day 6 or day 13, all nude mice displayed sudden and profound adverse reaction, evidenced by a fall in body weight, (in some cases equivalent to controlled deprivation of milk and maternal care), weakness and often death.

In one case however (Fig. 12), 13 day old nude pups experienced a rise in body weight, signifying a beneficial response with increased availability of milk but this was soon followed by an attenuated curve, then a fall in body weight. Death followed within 5 days.

The rate of fall in body weight in this case was very similar to the rate observed when 13 day old hairy mice were removed from the maternal nest and kept in isolation (Fig. 13), and suggests that the nudes were not feeding from the mother.

In this latter experiment, prior to removal of the hairy pups at day 13 groups of mice were gaining weight at the rate of 0.5g/mouse/day. This, therefore, was an example of a relatively efficient suckling situation, particularly in the case of the nude pups, although the differential weight between groups was preserved, i.e. the nudes weighed approximately 50% less than the hairy sibs. However, following removal of these hairy pups, the suckling efficiency of the nude animals was greatly reduced and although viability of nudes was maintained, their mean increase in body weight during the following 14 days was reduced to less than 0.1g/mouse/day. Their final body weight at weaning was some 44% less than the standard curve for nude mice in the general breeding colony. The very good level of nutrition obtained early post-partum, (in the presence of hairy pups), obviously influenced their later successful development.

The final litter manipulation experiments, when hairy pups were removed from the maternal nest, maintained in isolation for up to 3 days and then returned to the nest, were particularly informative. In these 2 experiments, prior to removal of some of the hairy pups, both hairy and nude pups were experiencing normal development, in terms of body weight v age.

In the case of another litter, where pups were removed at a later date post-partum, i.e. on day 10, a similar result occurred. The control hairy pups however, after 3 days isolation still weighed

slightly more than the most viable nude pup left with the mother

In both experiments, the following developmental sequence occurred, when hairy pups were returned to the nest. The deprived hairy pups experienced a period of re-adaption; in the case of pups deprived at day 5, this lasted 3 days during which time the pups remained at a stable body weight. The more mature pups of the other experiment, at 16 days of age, required only 1 day of similar re-stabilisation, before active suckling and rapid weight gain ensued.

The subsequent life history for nudes from both litters followed a similar course. The peak body weight achieved by the most viable nude was reached during the stabilisation period of the deprived hairy animals. As the hairy pups thrived, then the nude pups entered a period of decline in body weight, which ended in death in these cases.

Handling by the experimenter has been shown to have both positive and negative effects upon weight increase in young rodents as reviewed by Porter and Festing (1969). They reported that in handling young mice daily from birth to weaning, experimental pups weighed 0.5g less per mouse than controls. In other experiments daily stroking of rats from 0 - 21 days resulted in no difference in weight whilst other studies showed that handled mice weighed more than unhandled mice.

During studies into the economic production of uniform mice, Smith and Ryle (1968) pooled mice on the day of birth and then returned them to their own mother or put them into a foster litter. Of 1,417 young, only 8.5% of mice died before weaning. Rarely more than one per litter was lost and there were very few entire litters destroyed. Thus the handling appeared to cause no general disturbance of maternal behaviour.

In this nude mouse study, consideration was given to the possibility of handling effects and similar experiments were done where pups were first weighed early, in one case (day 5) and then later in another (day 10). Although the results are based on few experiments only, the resultant growth curves were similar in both cases, up to the point when litter manipulation experiments were began.

Altman and Sudarshan (1975) made a detailed analysis of the postnatal locomotion development of the laboratory rat; the three phases of pivoting, crawling and walking were reflected in quantitative data on the number of squares crossed in an open field.

During the first week minimal crossings were obtained, corresponding to the phase of development dominated by pivoting. During the second week there was a steady but low level of ambulation as crawling began, followed by a sudden increase in the level of efficient locomotion of the third week as walking and running actions developed. To what extent other factors such as eye opening contributed to the increase in exploratory activity in their subjects was not ascertained.

In this nude mouse study, the development of locomotion occurred over a much shorter time scale i.e. from about day 6 to day 12. The nude pups developed very slowly until day 12, then the mean number of squares crossed increased to 24. Hairy pups were producing this score by about day 9 and by day 12 this score had almost tripled to 69.

From two - three weeks of age hairy mice in a home cage situation were commonly observed exploring the complete cage area, returning to the nest and then setting out to investigate the food/water containers and wire top, etc. Nude mice of similar age seldom moved actively and remained mostly within the nest area.

Young mice tend to move in a circular crawling motion when placed in an open field. Thus a low score results as the mouse does not leave the starting square easily. When nude mice did begin to move they displayed this action more often and at a later age than the hairy mice, who soon developed elevated quadrupedal locomotion.

From a functional point of view the early maturation of fore-limb co-ordination can be related to the vital necessity of locating and maintaining a suckling station; forward progression is not essential at this stage of development as the mother tends to retrieve the stray young. However, as nude mice of 6 or 7 days of age were hardly able to score at all compared with hairy mice, it would appear that maturation of fore-limb co-ordination may be severely depressed, adding further weight to the evidence for reduced suckling ability in these animals.

In experiments where development of cerebellar cortex is selectively retarded during infancy, by focal exposure to x-rays, locomotor skills are affected, both in infancy and adulthood. Similar deficits were also observed by Altman, following severe undernutrition during the suckling period.

Fox (1965) discussed the work of MacDowell on the development of mouse runts. At birth they were small and hair growth was retarded; the eyes opened late (day 15-17) and the pups exhibited weak body

righting and locomotion. Primitive reflexes continued into post-natal development and at day 18 weak responses indicated that muscular weakness was coupled with delayed adult behaviour patterns. In the case of premature litters, the offspring were often smaller than normal and some mothers did not nurse such litters, which subsequently died. Other females showed normal maternal activity and reared litters that displayed a lag of 1 or 2 days only in development of their reflex responses.

Such a situation could be occurring in the case of nude mice where lack of physical co-ordination of the pups coupled with early disruption of the mother-young interrelationship (Rosenblatt, 1975) results in deprivation and undernutrition of nude offspring. Nude pups often display obvious muscular weakness, appear retarded in their behaviour patterns and are liable to die before maturity.

The high level of neonatal mortality affecting some nude pups has yet to be explained. Glucose is probably very important in the nourishment of nervous tissues, the activity and glucose uptake of which increases after birth, at least in the newborn rat, (Mandel and Weill, 1954). It is well known that small newborn mammals quickly develop hypoglycaemia if they are not fed; for example the blood glucose level of day-old rabbits, which have not been fed is less than half that of fed animals of the same age, (Hardman and Hull, 1969). Similarly, Mourek et al. (1964) found that in the newborn rat even a short fast lowers the blood glucose to a great extent and the low level may last for several days, even after feeding, due to a decreased capacity for gluconeogenesis.

Impaired nourishment of nude mice in early life could be partially responsible for the poor locomotor responses and feeding behaviour observed later on. If insufficient glycogen stores are then accumulated, further feeding problems occurring towards the end of the second week of life together with a change in maternal nursing behaviour and possible natural reduction in supply of the mother's milk could precipitate a further hypoglycaemic condition in nude pups resulting in weakness and severe locomotor and suckling dysfunction, from which they are unable to recover. It is apparent from this study that the cannibalism referred to in previous reports, probably occurs after pups have died due to nutritional problems, rather than as a result of direct infanticide behaviour from the adult parent.

In studying 15 different mouse strains, Thompson (1953) found striking differences between some of the strains in activity or 'exploratory behaviour'. This trait was measured empirically - in terms of distance traversed by an adult mouse in an unfamiliar situation during a standard test period. Some strains were found to be highly exploratory covering a considerable distance during the test while other strains hardly moved from the starting position.

Many other types of test have subsequently been used to quantify animal activity and were reviewed by McClearn (1964). In particular, Denenberg et al. described an experiment in which certain mutant mouse strains, viz pintale, hairless, pale-ear and short ear were tested against controls in a variety of situations, including open field, swimming test and shock escape test. Hairless mice were found to be significantly less active than controls in the open field and were notably poorer swimmers but did not differ on other measures.

The nude mouse results suggest that nudes do not move in an open field as actively as genetically related hairy mice. The results for the ten litters tested showed a highly significant difference between groups, both in the total number of squares crossed during a five minute test period and in the manner that test animals explored.

The hairy animals commenced movement within a few moments of being placed within the starting square. The rate of movement per minute increased very rapidly reaching a peak at 3 minutes of test. Thereafter activity level per minute fell slightly and began to plateau by the end of the test.

Nude pups were very slow to commence movement from the starting square. They peered around the barriers before emerging into a new square. A comparison of the nude activity rate with hairy controls shows clearly that the nude pups slowly increased their score in relation to the period of test, until by the end of the test period (5 minutes) they had just reached the activity level of hairy pups after only one minute of testing.

Archer's comprehensive review of tests for emotionality in rats and mice (Archer 1973) discussed the validity of such tests in experimental studies of rodent behaviour. Certainly, the enormous literature evidence citing defaecation as an indicator of emotionality is conflicting and may show little relationship to supposed measures of emotionality taken in other types of test, e.g. emergence tests, and active avoidance learning. Accordingly, defaecation characteristics in this nude mouse study were not analysed.

'Freezing' is often regarded as a direct measure of emotionality, (e.g. Denenberg, 1968), although Smith (1972b) found that it was not related to ambulation and negatively related to defaecations for mice. Certainly, nude mice were prone to stopping suddenly in the open field test, remained static for long periods and hence reduced their overall score.

The finding that the rate of ambulation for nudes increased with time in the test apparatus suggests that these animals were adapting to the novel situation over a period of time. It is significant that both hairless mice and nude mice should both display reduced activity in the open field test when compared with hairy controls and is useful evidence that the altered behaviour patterns displayed by nude mice are probably not mediated by hormonal factors related to their congenital athymia.

Two important factors which relate to the successful development of an animal at weaning are nutrition and stimulation in early life. Eichenwald and Fry (1969) reviewed the effects of nutrition on the learning process in man and animals. It is apparent that animals receiving inadequate calories and protein coinciding with the period in life in which the brain is growing most rapidly, produce a brain which is not only smaller at maturity than in control animals but also one which matures biochemically and functionally at a slower rate.

Such deprivation reduces the capacity of the experimental animal to learn at a later age in a similar way to rats born of and suckled by malnourished mothers, (Eichenwald and Fry, 1969). Similarly Hall (1979) showed that rats who were isolated in infancy showed reduced success in performance learning tasks later on, whilst rats given exposure to

stimuli when still immature showed positive transfer to discrimination learning in adulthood.

Subjects that startle less readily, that 'freeze-up' less, that do not show fear stimuli are the ones most apt to investigate and explore. This has been established in rodents; in these species reactivity may be increased, while the exploratory tendency is decreased by restriction of stimulation in infancy, that is, by solitary rearing conditions and a generally monotonous early environment (Sluckin, 1970).

The history of development detailed in this present study suggests that the female may 'handle' nude pups in a similar manner to hairy pups; she covers them (to a greater or lesser degree) with her body and licks and retrieves them. However, quite obvious differences in the subsequent periods of activity, exploratory/locomotion behaviour and pup-pup interaction experienced by the two pup groups was found.

Hairy sibs soon leave the nest and begin to interact with each other and their environment. Even by day 21 however, some nudes were spending much of the observation time still in the nest and if they did leave the nest, were often retrieved by one or other parent. Such deprivation affecting early social contact and perceptual development may have repercussions later in life for homozygous nude mice, affecting not only exploratory behaviour but also sexual activities (Flanagan, 1966) and maternal behaviour (Festing and King, 1974).

FURTHER RESEARCH NEEDED

Of the 443 publications listed by the Nude Mouse Secretariat in 1977, only 13 were directly concerned with breeding and husbandry studies in nude mice and little reference has been made to behavioural development. The results of this present study probably pose more questions than they manage to answer; as a broad qualitative rather than quantitative investigation, I have attempted to give an overall account of nude mouse development. In many areas detailed statistical analysis is lacking as the number of original experiments undertaken have been necessarily limited.

The behaviour scan studies suggest that the mother undergoes normal pre and post-partum development in terms of physiological and behavioural development. Probably, the most important maternal function requiring further investigation is that of nursing/suckling. Successful intake of milk by the pup relies on a close physical relationship between mother and offspring:

- i. The mother must take up a suitable position over the infant.
- ii. The infant must locate the mammary area.
- iii. The infant must initiate suckling by grasping the teat in its mouth and by stimulating milk ejection
- iv. The pup must generate a negative (sucking) pressure.
- v. The offspring must stimulate the mother to induce mother-young interaction and a natural development of behaviour between the two.

A more detailed investigation of nursing and suckling behaviour using improved techniques may reveal the true nature of suckling

difficulties and at what stage (i. - v. above) they develop and confirm the existence of a 'critical suckling period' at day 10 - 13 post-partum.

The major difficulty is one of technique: the mother and pups are screened by bedding material and the mother covers the pups with her body. Even the use of transparent cages, viewed through an adjacent observation panel, as in this study, is not a perfect technique. The use of carefully positioned video recording cameras would facilitate more detailed recordings from various nest angles.

Further work should be undertaken to confirm the reasons for the suckling difficulties observed in this and other reports. The studies by Teicher and Blass (1976) Hofer et al. (1976) and Moltz and Leihdahl (1977), show that specific pheromones may be essential in the rat, for the young to locate the lactating mother and thus initiate suckling. Further studies on suckling behaviour should therefore include a comparison of nudes with hairy controls with respect to the action of pheromones.

The amount of milk available in the rat as a result of passive withdrawal is small; milk ejection therefore, controlled by oxytocin, is important in the interactions of mother and litter. Milk ejection must take place before the suckled young can drink milk in any substantial quantities; it is a response to the suckling of the pups which then itself acts as a new stimulus, changing the behaviour of the pups (Drewett and Trew, 1978).

Rat pups can detect the occurrence of milk ejection at the nipple even when the flow of milk is prevented by ligation of the milk duct and this may be due to an olfactory cue derived from a substance

secreted in the nipple area, in response to oxytocin. Can mice, and nude mice in particular, respond to such a cue, to stimulate oxytocin release and milk ejection?

In the investigation of ultrasonic calling too few pups were available for statistical tests of the differences between nudes and others but the suggestion that there may be a difference between the groups is very interesting as at day 10 it would be expected that the nude would be most sensitive to a lowering of body temperature as they begin to regulate their own body temperature for the first time since birth. The final stage in the development of homiothermy lasts from day 14 - day 19 and this may explain why the recordings on day 17 were almost nil.

The finding that nude pups may call more times and for longer must be confirmed by repeat experiments. If nudes do react differently in this way, it could explain why the mother tends to retrieve nude pups more actively than hairy pups, when they are both isolated from the nest although the possible influence of pheromones must also be considered more fully. Elwood (1975) noted that gerbil mothers would leave pups outside the nest for up to 1 hour before retrieval occurred. Does the nude mouse get returned to the nest in a shorter time? - is this related to ultrasonic calling?

Extremely valuable information may result from a study of homozygous females, who will produce nude offspring only. In the resultant high mortality rate (reaching 100% within 2-3 days) due to inadequacy of the mother or inability of pups to suckle? Would the introduction of similar aged hairy pups result in more efficient nutrition and a measurable weight increase for nudes, albeit insufficient for successful development? Also, does the nude female

exhibit fertility and maternal behaviour problems as a result of behavioural inadequacies or physiological dysfunction?

The investigations on the development of locomotion show that the behavioural scan results for nudes are mirrored by open field testing: nudes are slow to develop quadrupedal locomotion. Even when they develop into healthy weaned animals, nudes continue to display reduced activity in an open field. Would hairy mice subjected to early malnutrition and lack of maternal care score lower than controls kept with the mother?

An efficient immune system is essential for optimum growth and it appears that malnutrition may reduce antibody production and particularly cell-mediated immune responses (Halliday, 1980). Experimental infections in SPF animals cause a depression in growth, due to changes in absorptive capacity of the small intestines (Coates, 1980).

Also, natural stresses such as starvation and overcrowding increase corticosteroid output, further disturbing the immune system and rats often show reduced growth and a higher mortality when their intestines became increasingly sensitive to corticosteroids during the second week of life (Halliday, 1980).

The effects of depressed T-lymphocyte production in nude mice and the interaction of hormones and infection upon the immune system, in affecting growth cannot be ignored then even to some degree, in SPF or barrier maintained animals, and deserves particular investigation in relation to nude mouse development.

1



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Fig. 25. Comparative Nest Studies

1. Heterozygous mother and nude male parent with newborn litter. Note well-made, intact nest and apparently healthy suckling pups; male in the nest.
2. Homozygous parents with similar age litter. Nest is completely disrupted, and scattered around the box. The pups are hypothermic and moribund.

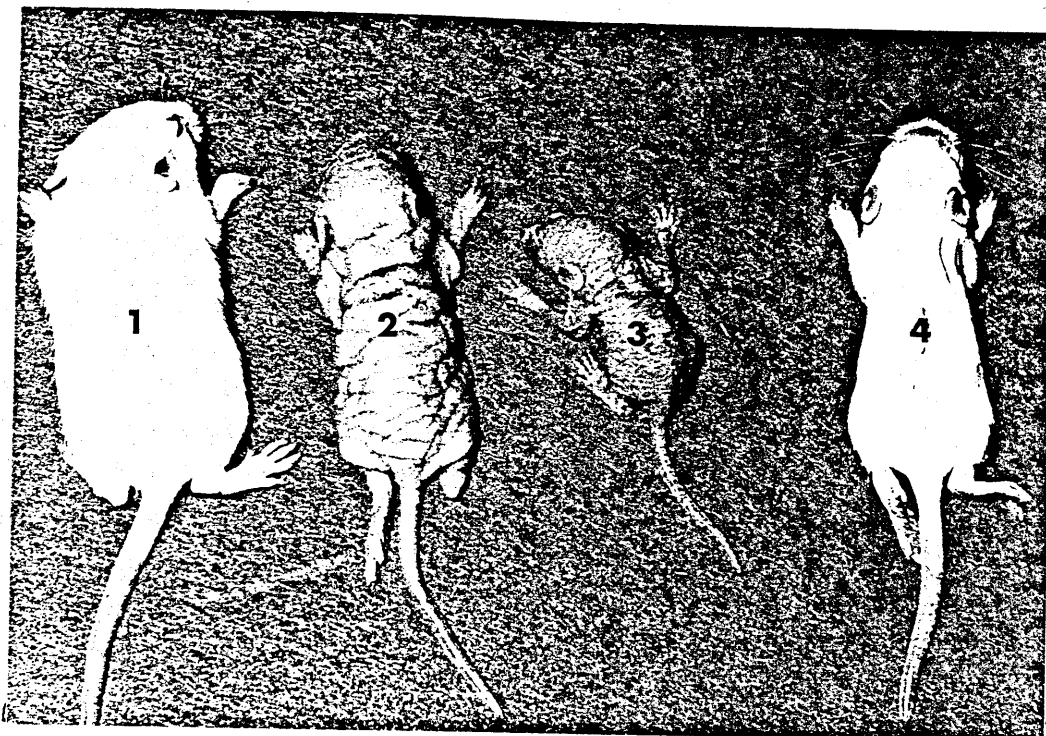


Fig. **26**. The Effect of Deprivation

Pups 1, 2, and 3 were maintained with the mother.

Pup 4 was isolated from day 7 - 10.

Result: Pup 4 weighed 4g less than the hairy sib (pup 1) that was kept with the mother.

Nude pups 2 and 3 displayed a similar differential, although both had been kept with the mother.

Does pup 4 display less activity in an open field at weaning, than pup 1? It is reduced to the level of nude mice?

Fig. 27. suggests the 'developmental pathways' that nude pups may follow during the postnatal period. Lack of adequate nutrition could be confirmed by comprehensive biochemical testing; insufficient fat and glycogen stores in early life may be a contributory factor to later hypoglycaemia and early death if suckling difficulties occur. As brain growth has a direct relationship to early nutrition, a study of nude mouse brain size and DNA content may confirm that early malnutrition does occur. Does the altered exploratory behaviour pattern in weaned animals then correlate with smaller brain size? Again, more intensive behaviour scans should help to identify the periods of deprivation more accurately. Controlled deprivation experiments using hairy pups may also yield interesting findings.

Some preliminary comparative studies using hr/hr hairless mice have been undertaken in conjunction with King's College Hospital Medical School London. Homozygous hairless mice developed in the normal way until approximately 14 days of age, at which time hr/hr mice begin to lose fur, (usually at the time the eyes open). By day 19-20 they are fully hairless.

Five litters were studied for measurement of productivity and body-weight increase. Preliminary results suggest that homozygous mice may be more likely than normal mice, to be still-born or to die before weaning. There was little difference between groups at 14 days of age (when weights were first recorded) but at weaning, the normal sibs weighed 24% more than hairless animals. More weights are needed to confirm whether or not hr/hr mice are already smaller than normal pups, before they become hairless.

With regard to crossing homozygous hr/hr animals, an accidental mating resulted in a good litter which was successfully reared. However, a subsequent study using 6 further monogamous pairs showed that the high perinatal mortality rate which resulted made it uneconomical to use this system, (Thatcher, 1980 - Personal Communication) Why then are homozygous hairless mice, like nudes, poor in maternal abilities?

A recent publication by Festing et al. (1978) announced the establishment of a breeding colony of nude rats. These animals carry a recessive mutant gene resulting in athymia and hairlessness. An original breeding colony had been maintained with difficulty until the early 1960's when it died out! However, the recessive mutant was apparently maintained at low gene frequency within the random-bred colony and homozygotes were recovered in 1975.

The nude rat presents as in the case of the nude mouse: bent and sparse vibrissae, absence of hair or sparse coat which is unevenly lost, and with an abnormal thymus rudiment. Growth of nude rats is reduced to about 60% - 80% of hairy littermates and pre-weaning mortality may be higher than for heterozygous sibs.

Here then, is a perfect model to confirm the findings of this study using another rodent subject displaying a similar mutation. Much of the literature has derived from rat rather than mouse studies, and some errors in comparison and extrapolation of results have possibly occurred; the use of this animal in repeat experiments would be of great comparative value.

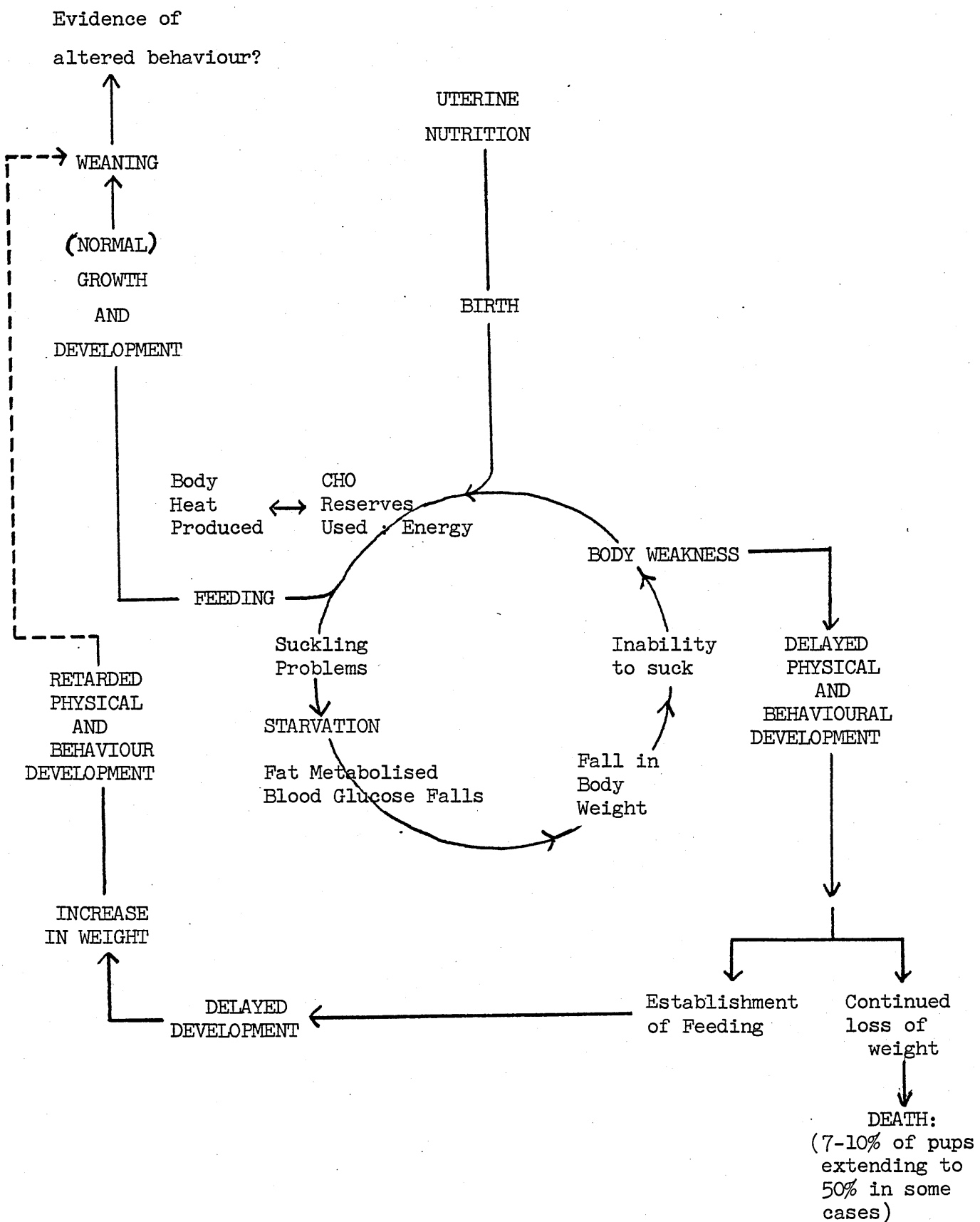


Fig. 27. Possible Development Pathways for Nude Mice

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APPENDIX 1

The following publications illustrate some of the research projects which were made possible at the Cambridge Veterinary School, as a result of the breeding colony of nude mice described in this work:

Publications 1975 - 81

Betton, G.R. (1975). The Biology and Immunology of Canine Melanoma. Ph.D. Dissertation, University of Cambridge.

Holmes, J. (1978). Aspects of an intranuclear isotope labelling technique for measuring tumour cell death in vivo and in vitro. Ph.D. Dissertation, University of Cambridge.

Holmes, J.M. (1981). Measurement of the rate of death of canine transmissible venereal tumour cells transplanted into dogs and nude mice. Res. Vet. Sci. 30, 248 - 250.

Morgan, D.R. (1976). Tissue culture and transplantation techniques used in the study of canine tumours. F.I.S.T. Dissertation, Institute of Science Technology.

Morgan, D.R. (1977). Observations on the breeding and maintenance of athymic nude mice. J. Inst. Anim. Tech. 28:83.

Morgan, D.R., Owen, L.N. and Onions, D.E. (1978). Growth of canine lymphosarcoma in x-irradiated and non-irradiated Athymic (nude) mice. Europ. J. Cancer, 14, 1353 - 1357.

Onions, D.E. (1976). Immunological and Aetiological Studies of Canine Lymphosarcoma. Ph.D. Dissertation, University of Cambridge.

Oughton, S.M.J. and Owen, L.N. (1974). Transplantation of Dog Neoplasms into the Mouse Mutant Nude. Res. Vet. Sci. 17, 414 - 416.

Owen, L.N. and Morgan, D.R. (1975). Growth of Canine Solid Mammary Carcinoma In Vitro and in Nude Mice. Abstract in Br. J. Cancer 32, 248.

Owen, L.N., Morgan, D.R. and Bostock, D.E., and Flemans R.J. (1976)

Tissue Culture and Transplantation Studies on Canine Mammary

Carcinoma. Europ. J. Cancer. 13, 1445 - 1449.

Owen, L.N. (1980). Transplantation of Canine tumours into

immunosuppressed dogs and nude mice. In Immunodeficient animals

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